



WORKSHOP ON MEMBRANE SCIENCE
USING X-RAY TECHNIQUES

August 16-18, 2004, Advanced Photon Source, Argonne National Laboratory

Biomembrane Problems Studied by X-ray Diffraction

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Applications to membrane problems

(0) Background—diffraction from lipid bilayers

(1) Gramicidin-a passive ion channel

- 1.1) Ion binding sites
- 1.2) Non-conducting state
- 1.3) Hydrophobic matching
- 1.4) Membrane-mediated protein-protein interactions

(2) Protein-lipid interactions--Hydrophobic Matching

(3) Antimicrobial peptides

- 3.1) Transmembrane pores
- 3.2) Membrane thinning effect
- 3.3) Pore structure

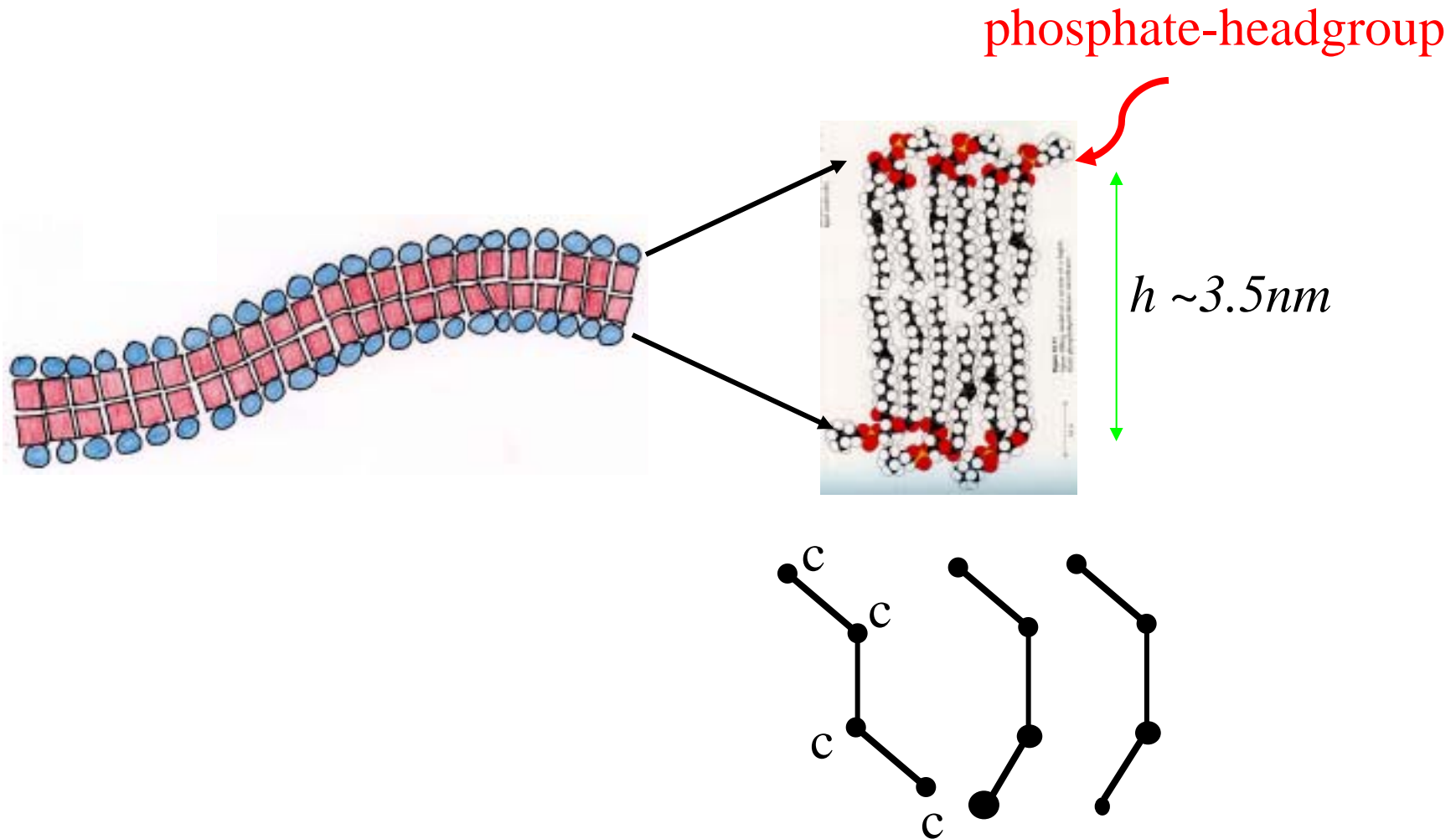
(4) Membrane fusion problems

- 4.1) Stalk intermediate state
- 4.2) Spontaneous curvatures of lipid mixtures

(5) Collective chain dynamics by inelastic x-ray scattering

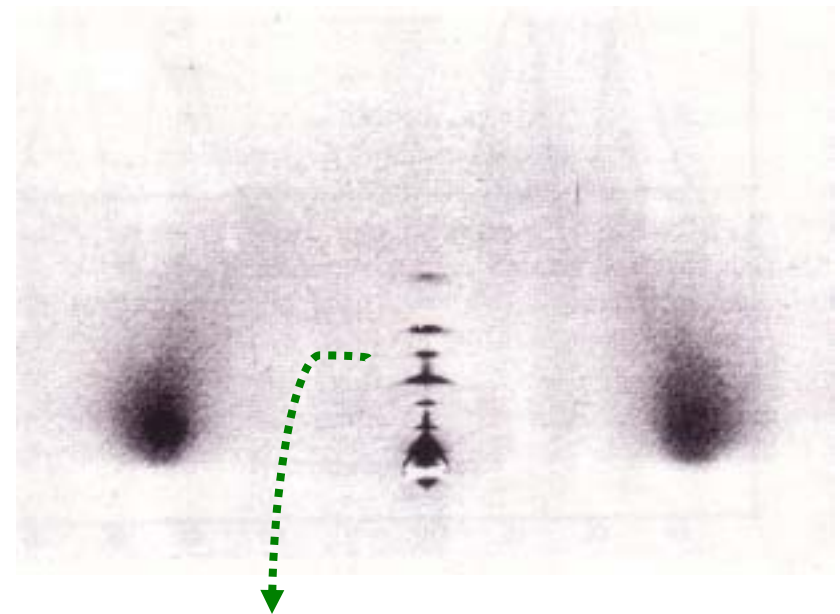
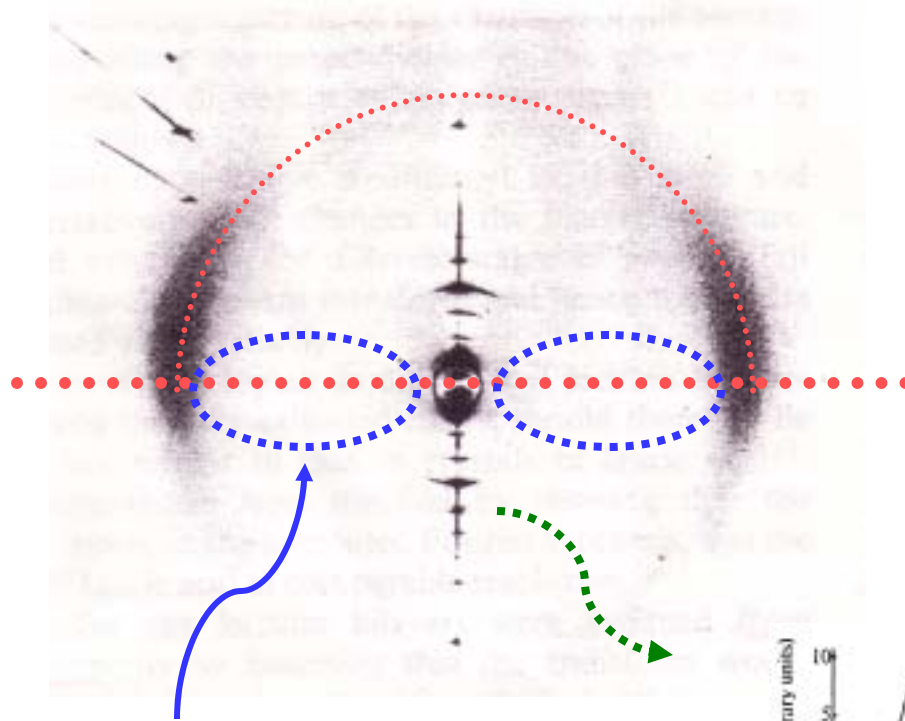
CONCLUSION

Lipids and Membranes

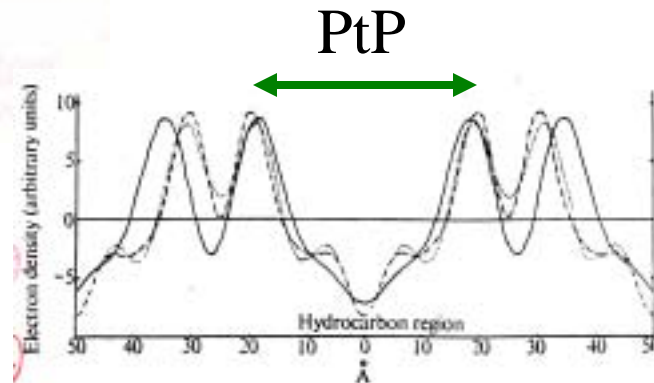


Levine and Wilkins, *Nature* 230, 69, 1971

Wilkins, Blaurock and Engelman, *Nature* 230, 72, 1971



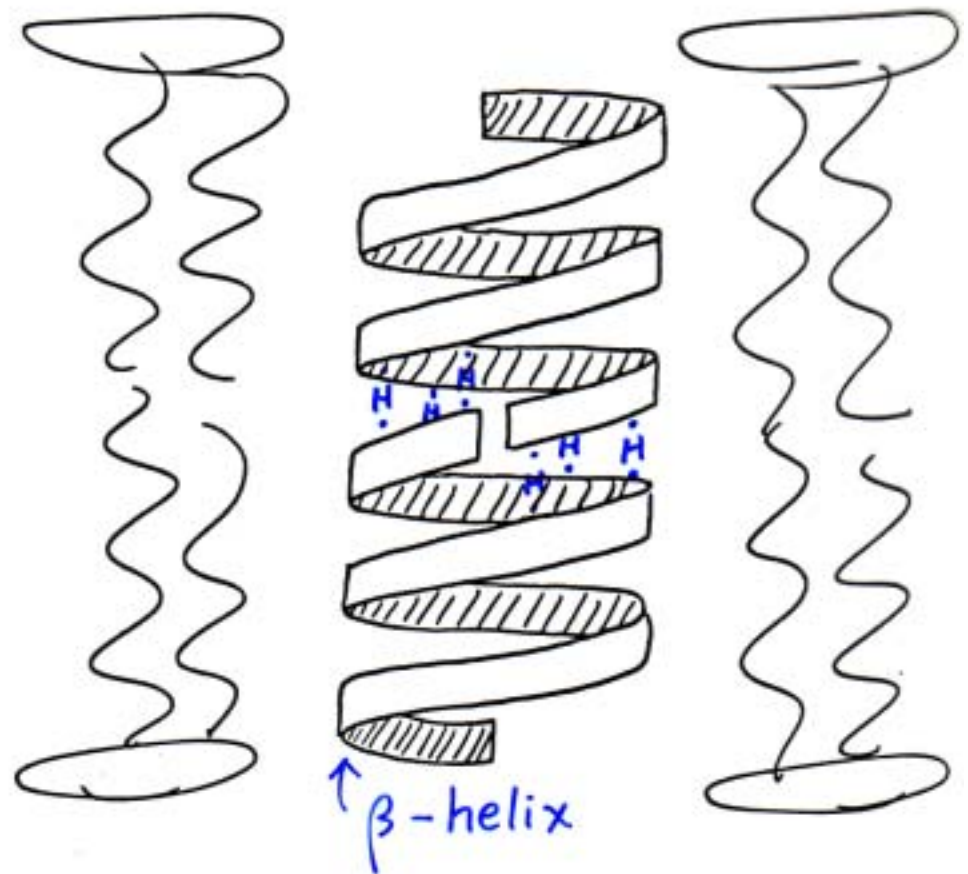
in-plane scattering



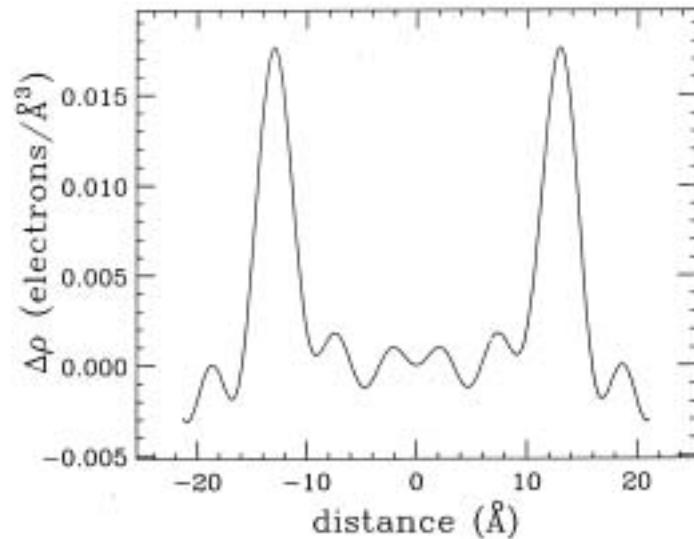
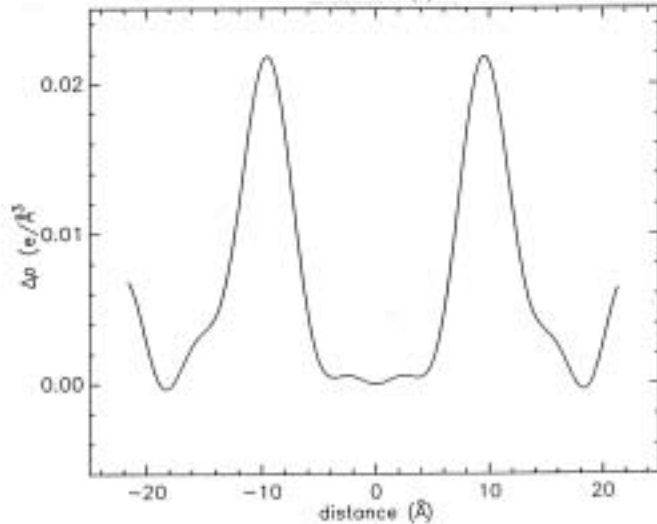
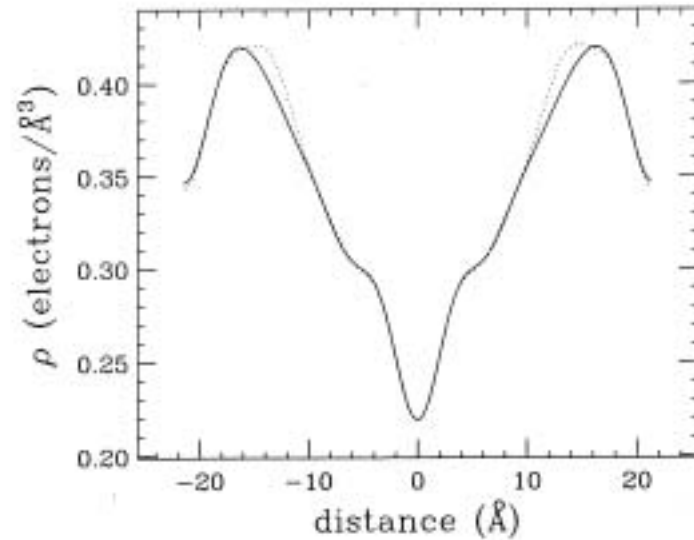
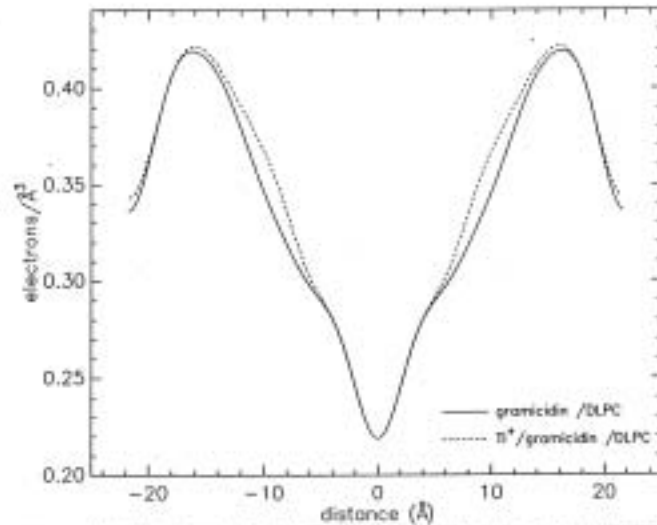
(1) Gramicidin

Gramicidin is a 15a.a. long L-D-L-D alternate peptide that forms a head-to-head dimeric passive ion channel. It has been used for a case study of ion transport across membranes.

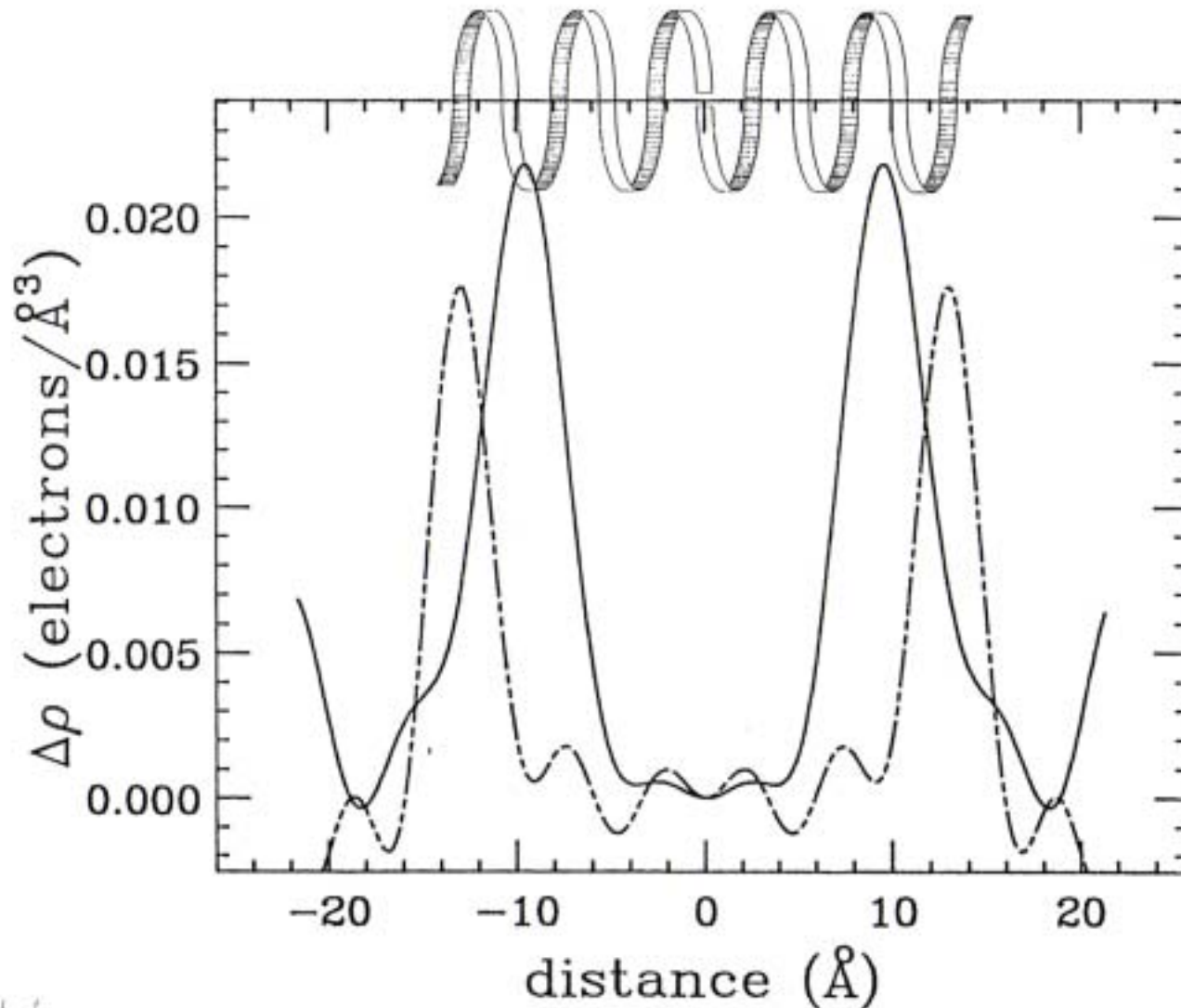
It has two ion binding sites, but they are not obvious from the molecular structure.



Electron density profiles by lamellar diffraction

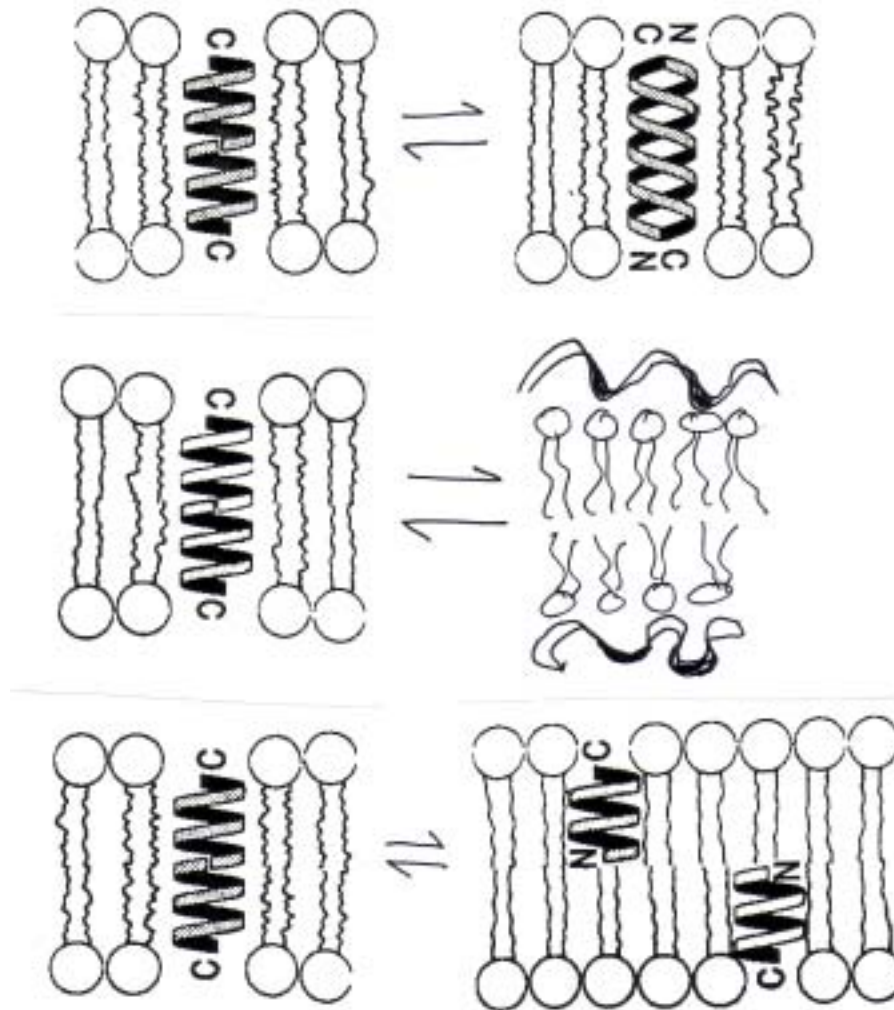


1.1) Location of ion-binding sites



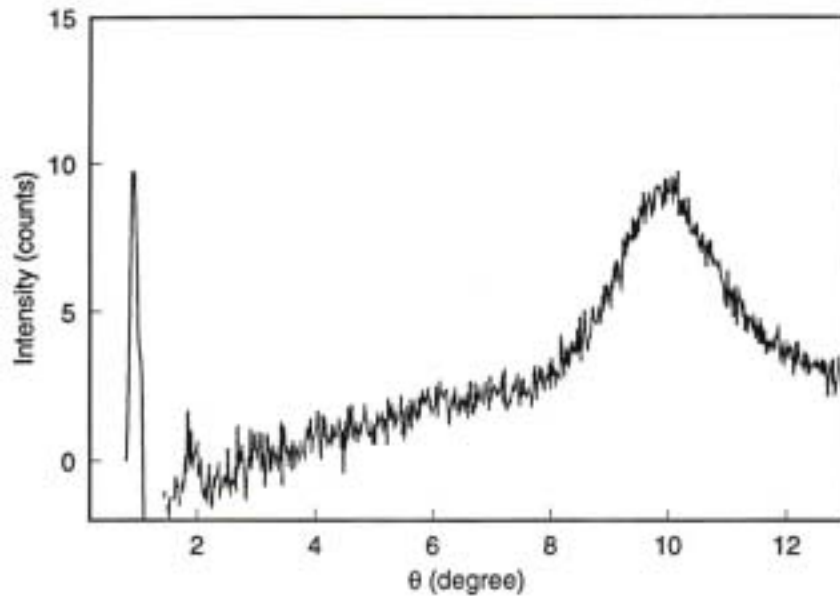
Olah et al.,
J. Mol. Biol.
218, 847
(1991)

1.2) What is the non-conduction state of gramicidin?

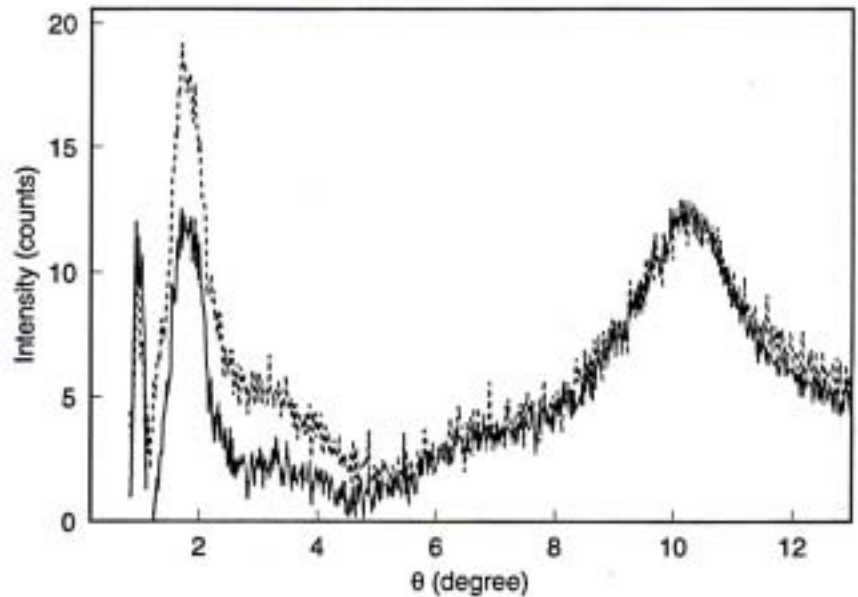


Answer: gramicidin monomers are beta-helices.

Pure DLPC



DLPC/GA and DLPC/G-boc



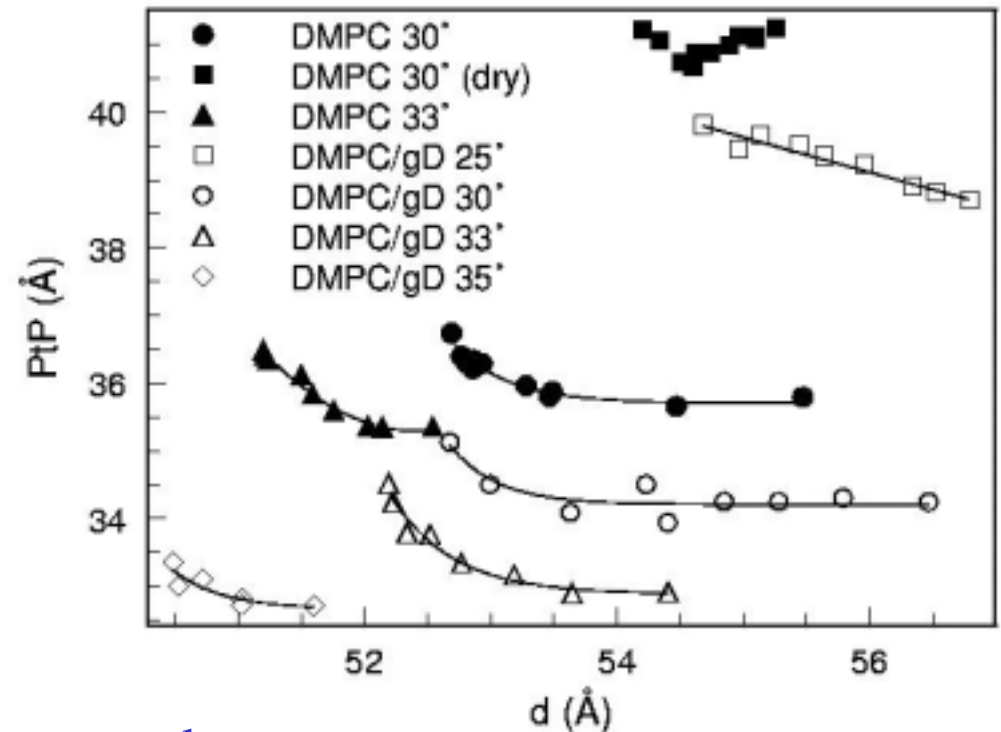
He et al., Biophys. Chem
49, 83 (1994)

1.3) Membrane thickness effect implies hydrophobic matching—a well accepted concept that has little direct evidence.

Huang.,
Biophys. J.
50, 1061 (1986)

In the fluid phase near full hydration:

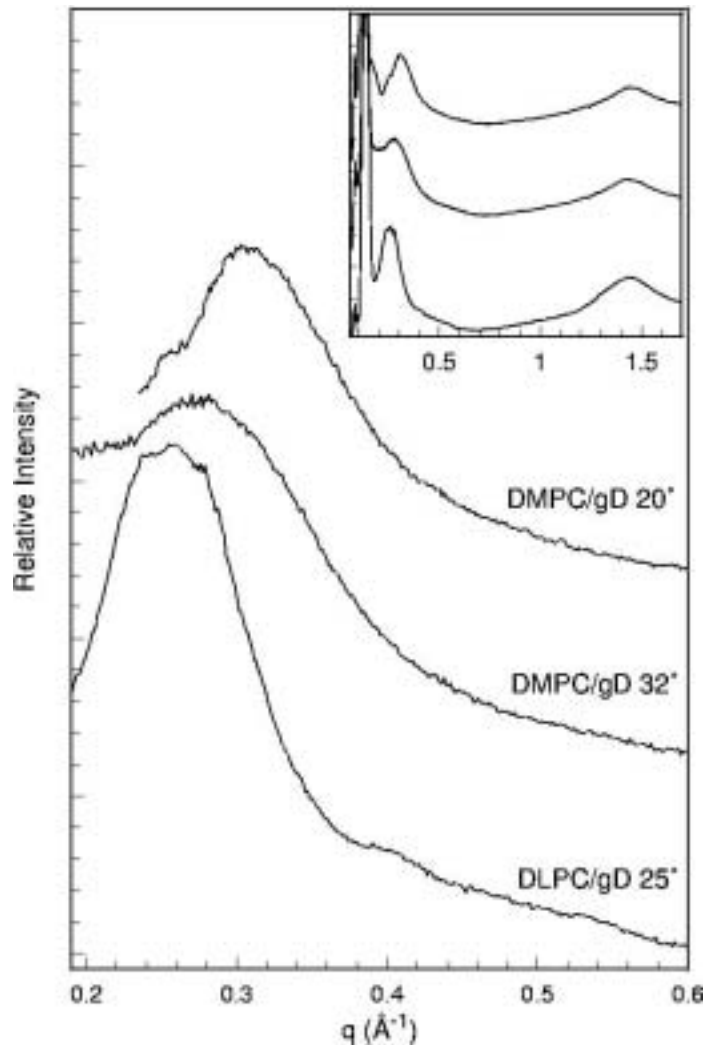
	PtP
pure DLPC	3.08nm
DLPC/GA	3.21nm
pure DMPC	3.52nm
DMPC/GA	3.27nm



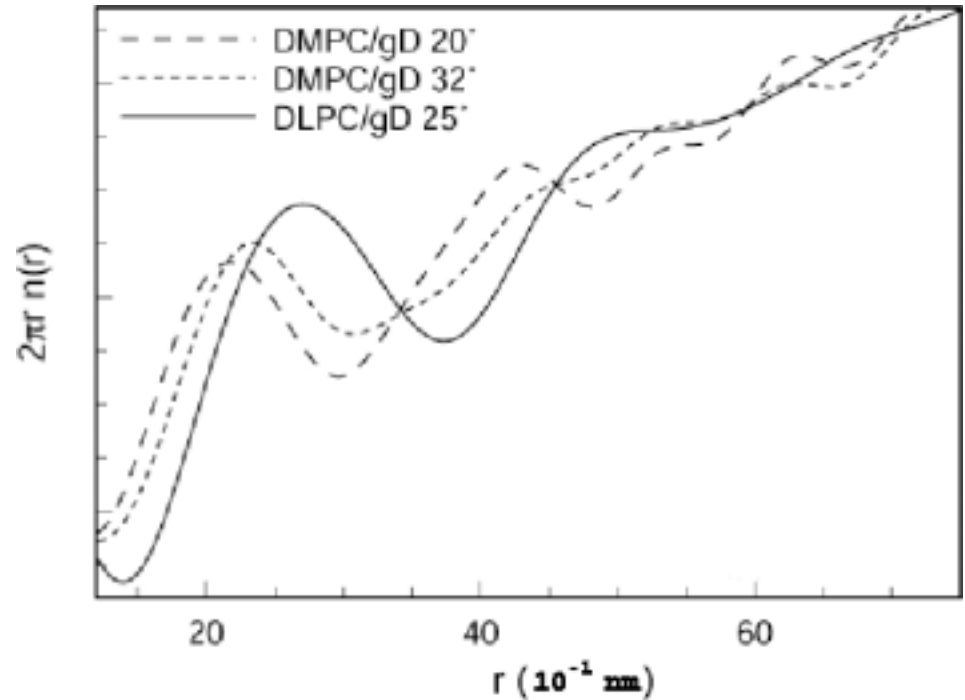
Harroun et al.,
Biophys. J.
76, 937 (1999)

1.4) Hydrophobic matching implies membrane-mediated protein interactions, another well accepted concept with little direct evidence (e.g., Engelman et al., 1983; Chan et al., 1983).

In-plane scattering



Radial distribution function of gramicidin in DMPC and DLPC

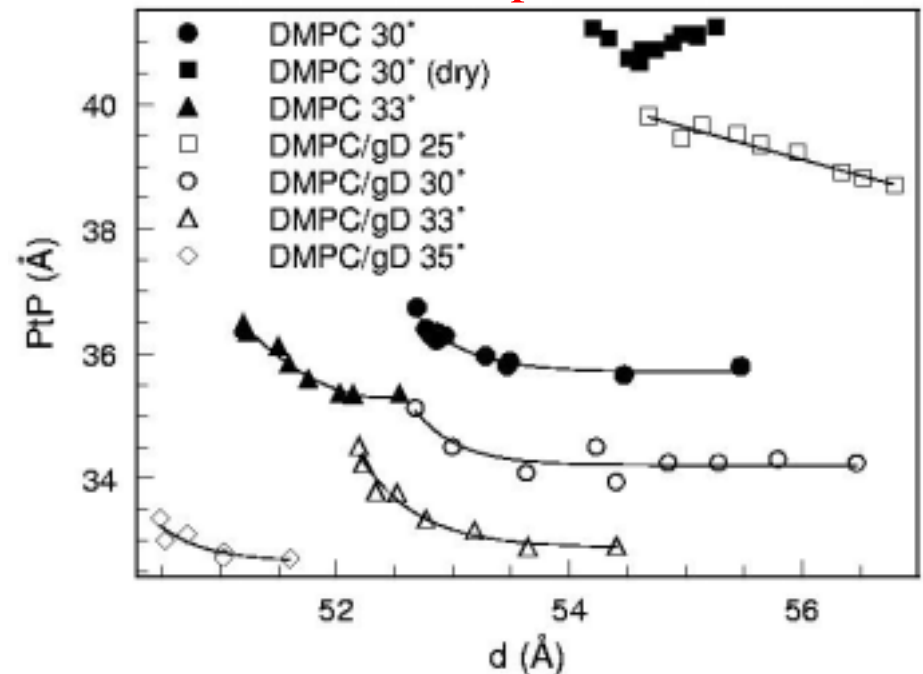
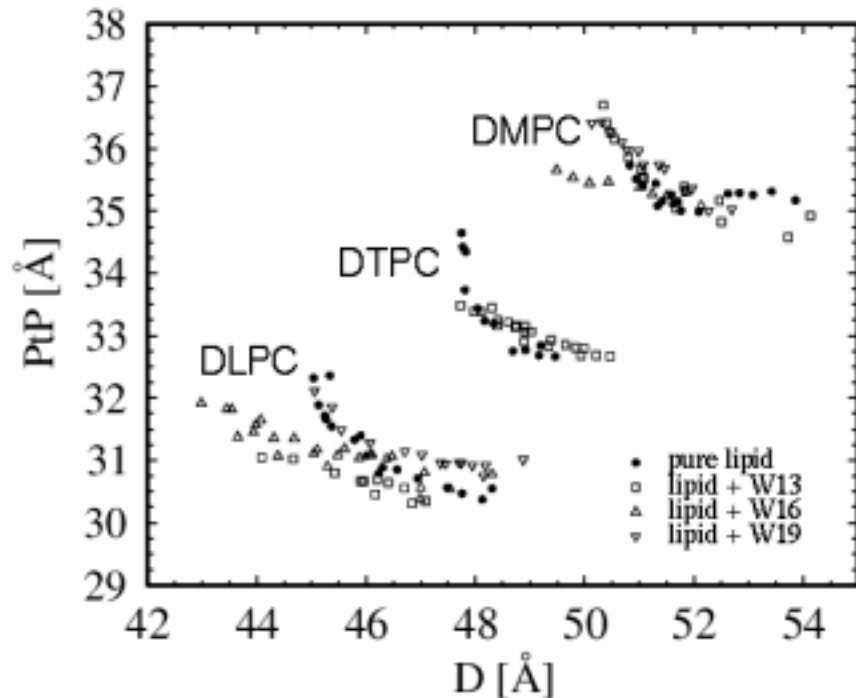


Harroun et al.,
Biophys. J. 76, 937 (1999)

(2) No hydrophobic matching for single helices!

WALP, a hydrophobic sequence of Leu and Lys of varying length, bordered at both ends by two Trps.

for comparison

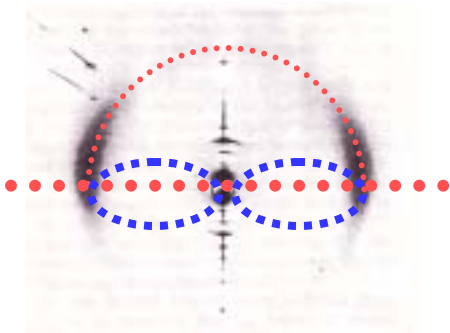


Weiss et al., Biophys. J. 84, 379 (2003)

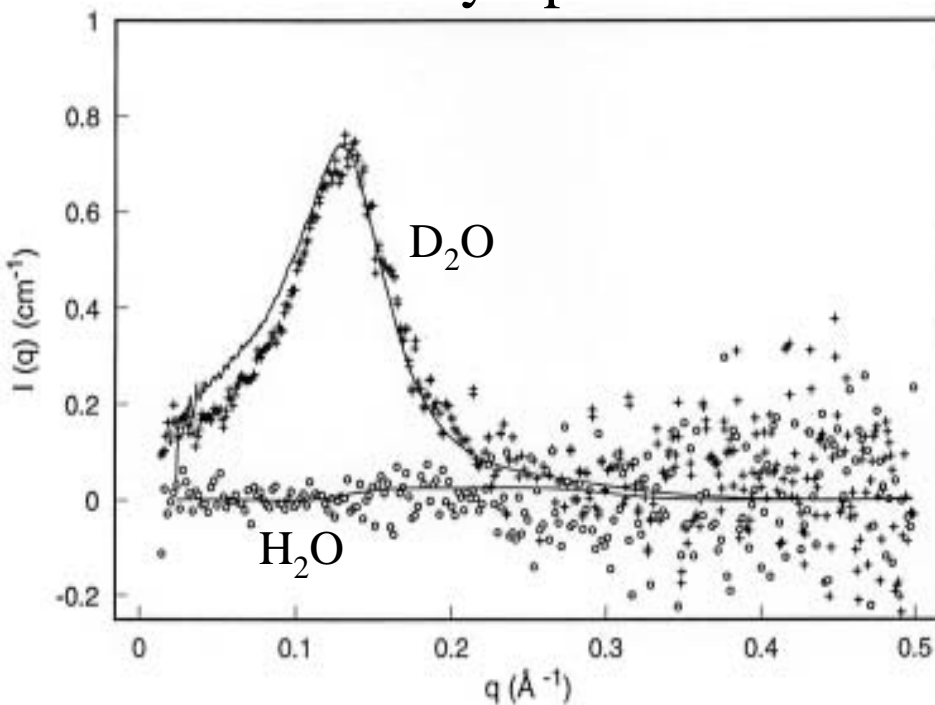
(3) Antimicrobial Peptides

3.1) Detecting transmembrane pores by neutron in-plane scattering

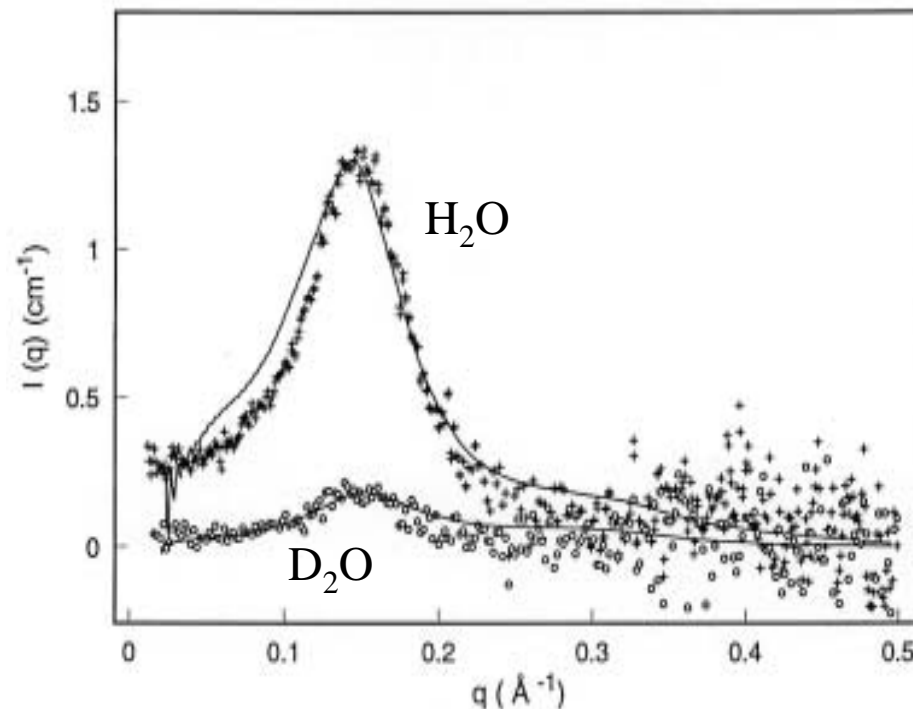
He et al., Biophys. J. 70, 2659 (1996)



ordinary lipid

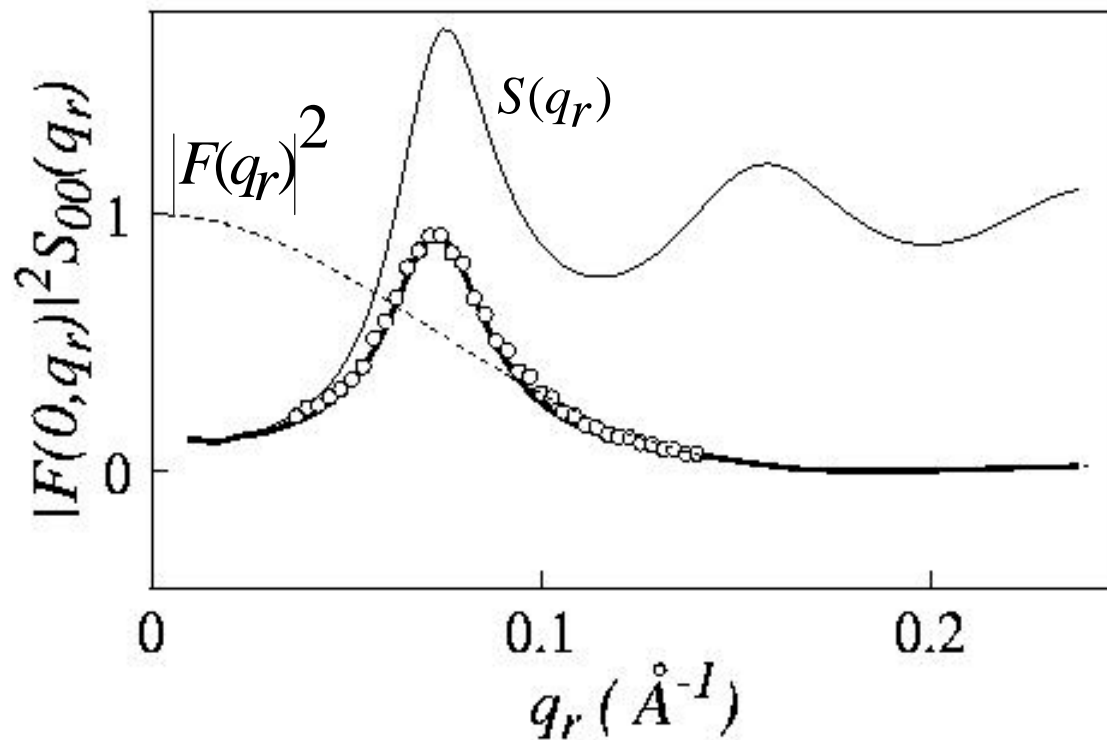


perdeuterated lipid



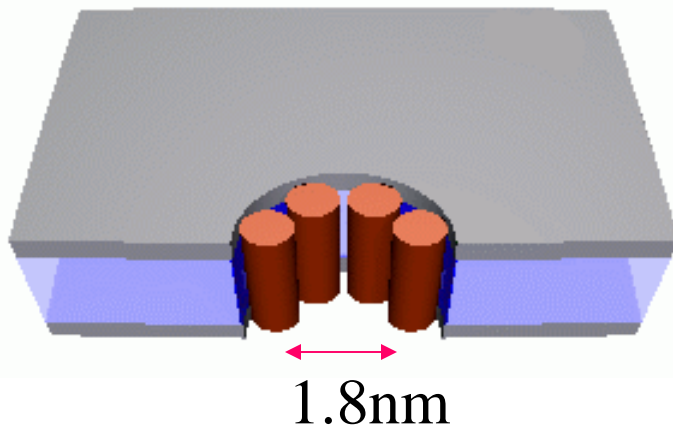
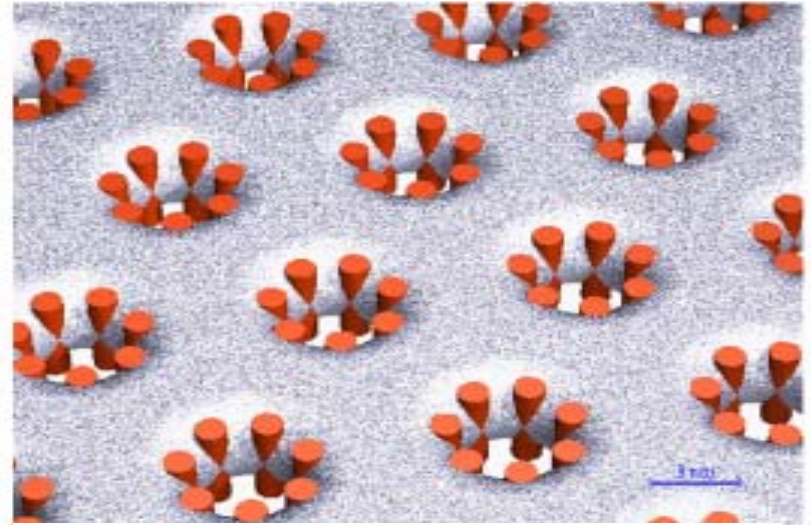
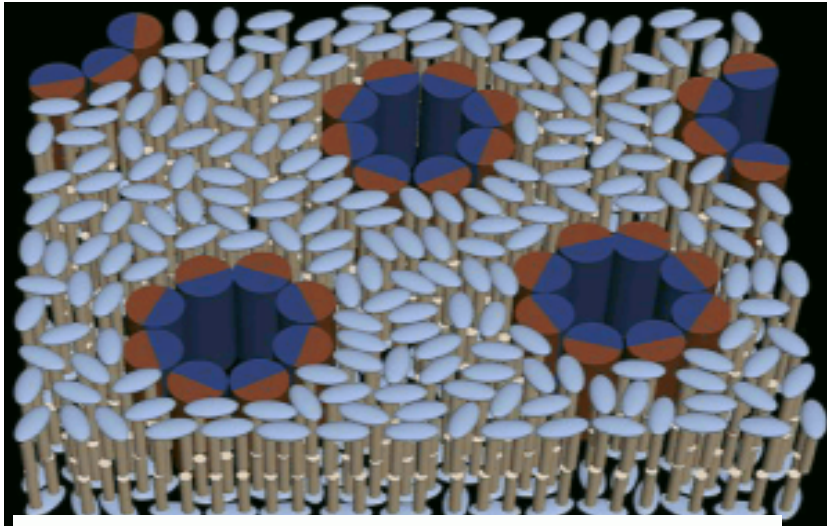
Analysis of neutron diffraction from fluid membranes

$$I = |F(q_r)|^2 S(q_r)$$

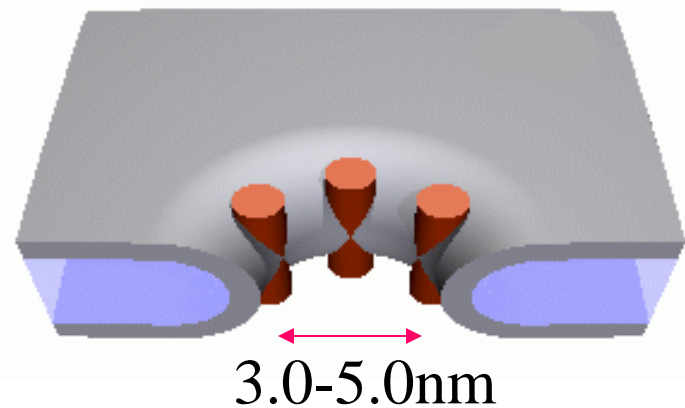


Putative structures of pores formed by antimicrobial peptides

Yang et al., Biophys. J. 81, 1475 (2001)

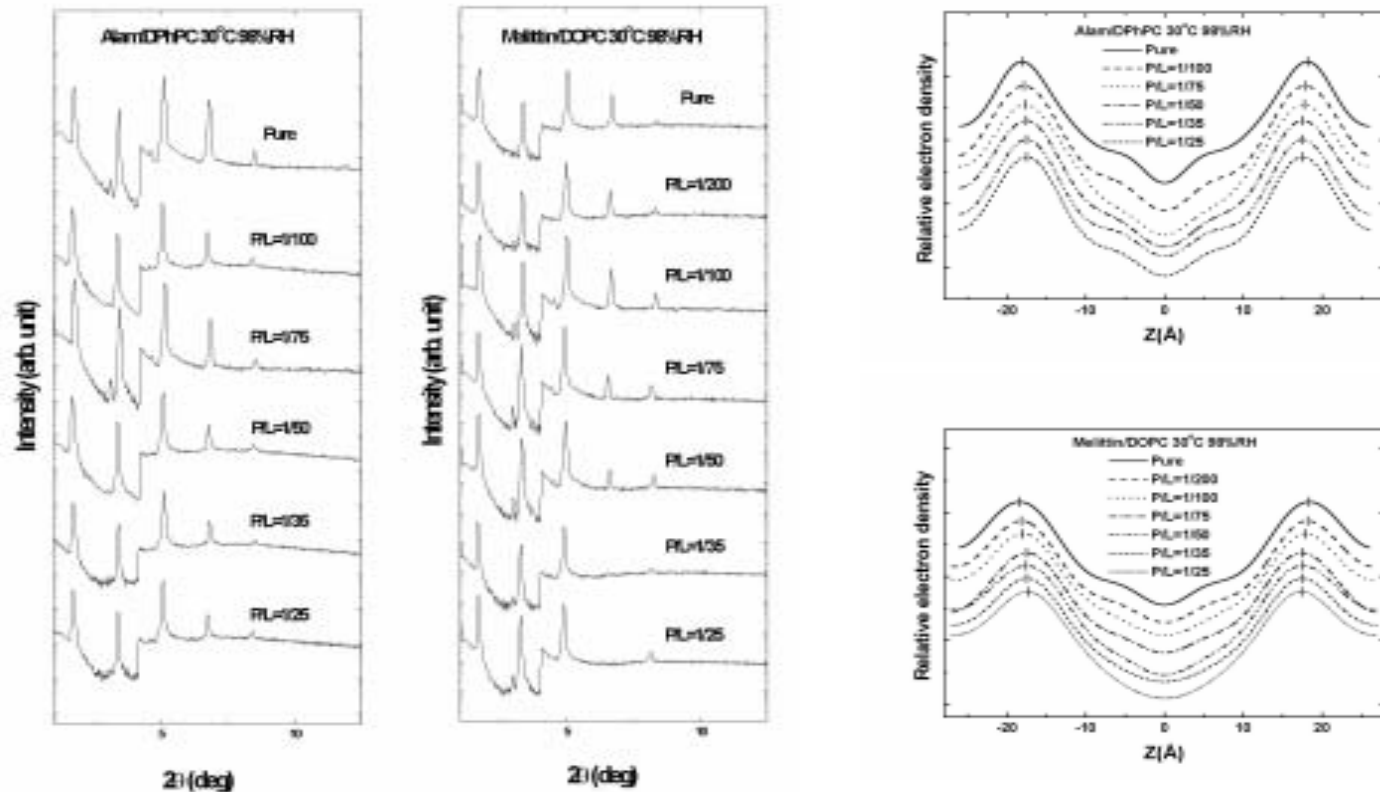


Barrel-stave model



Toroidal model

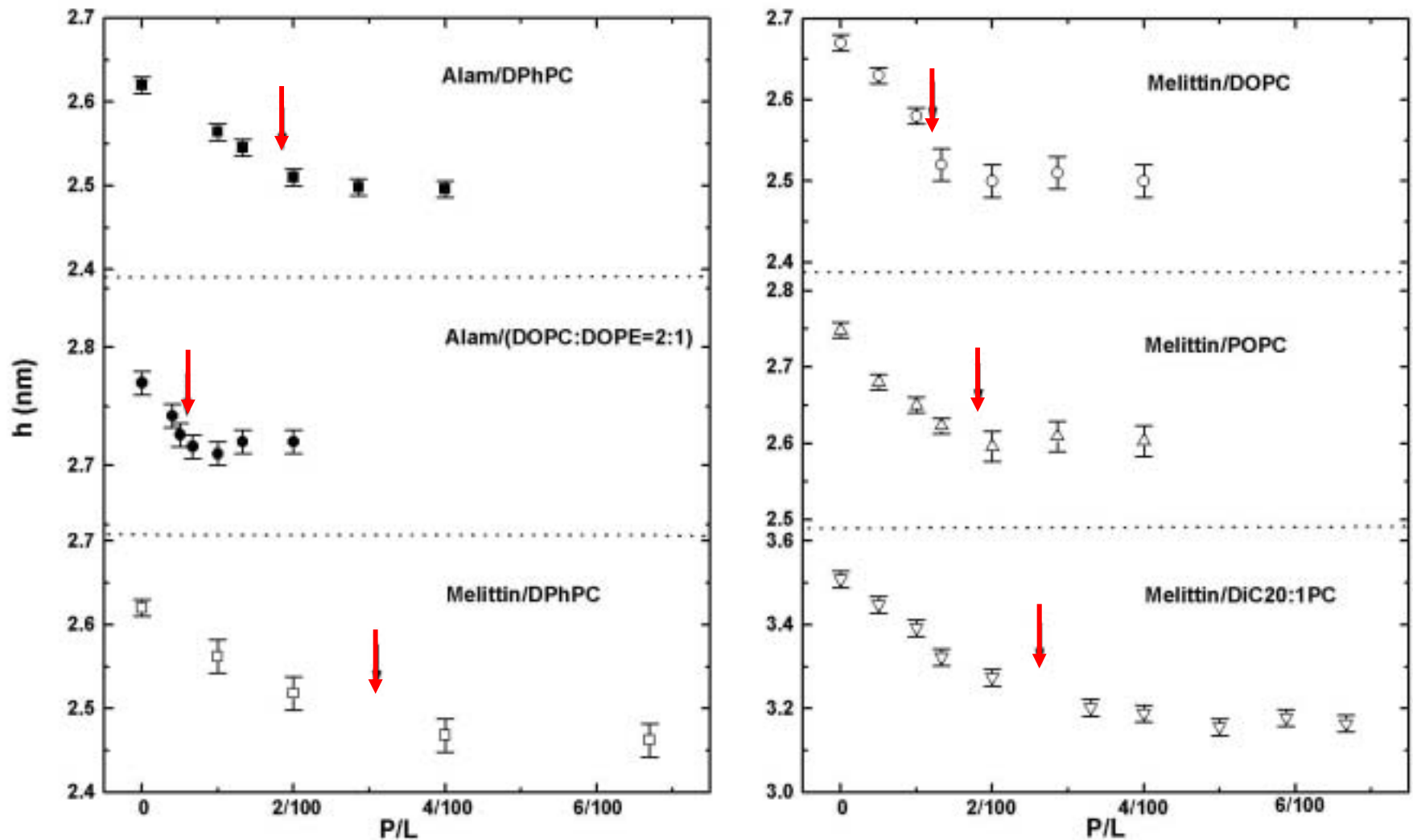
3.2) Pore formation can be seen as membrane-mediated two-state micellization. At low P/L, the peptides are monomers on the interface. At high P/L, pores are formed like micelles. The membrane mediation is observed in the thinning effect.



Membrane thinning

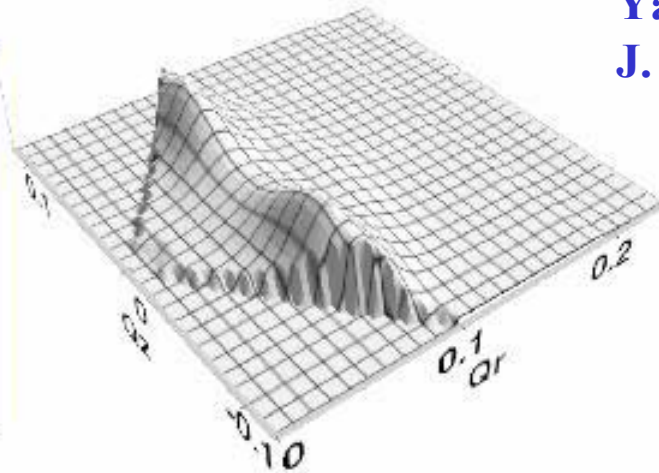
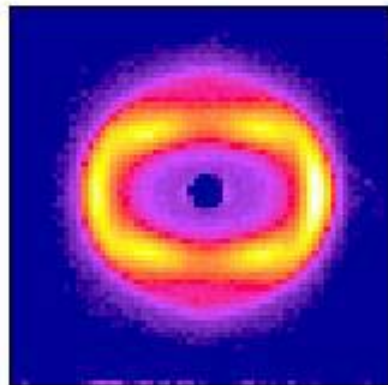
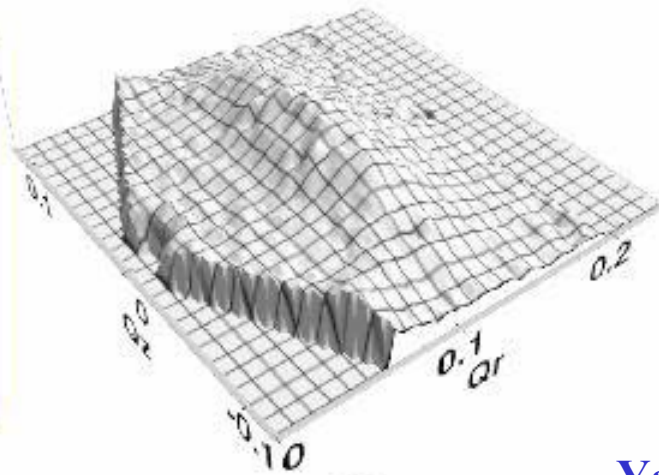
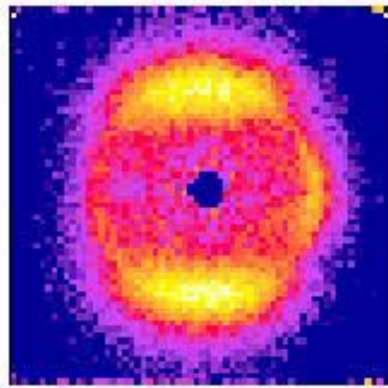
↓ threshold
by OCD

Chen et al., Biophys. J. 84, 3751 (2003)



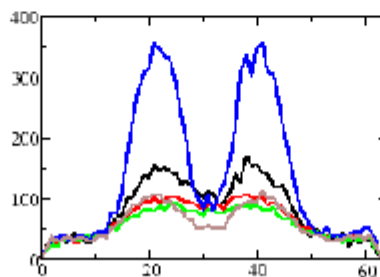
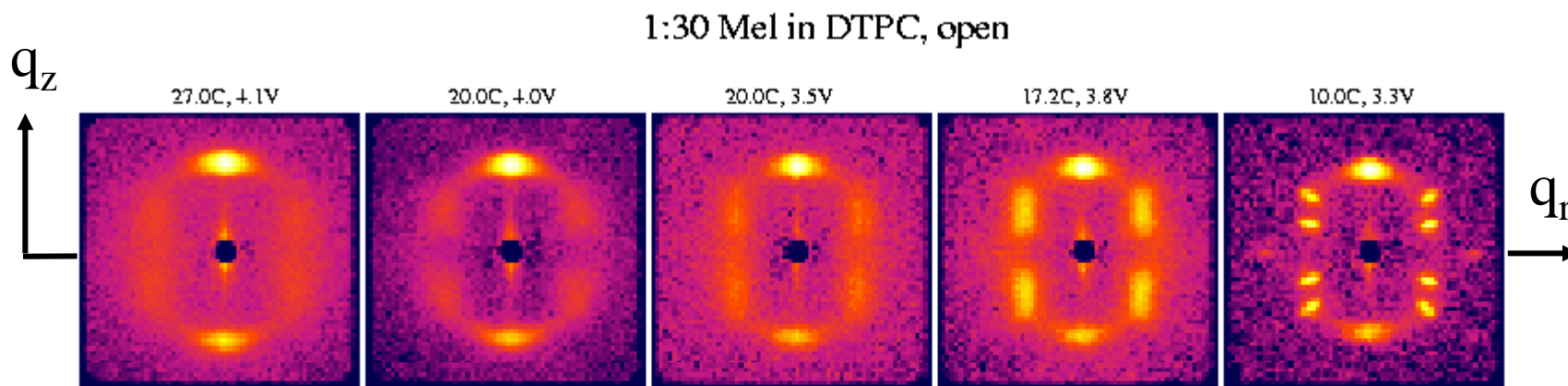
3.3) How to study the pore structure?

Induce inter-bilayer correlations.



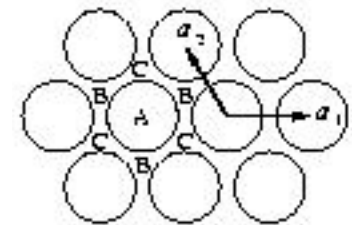
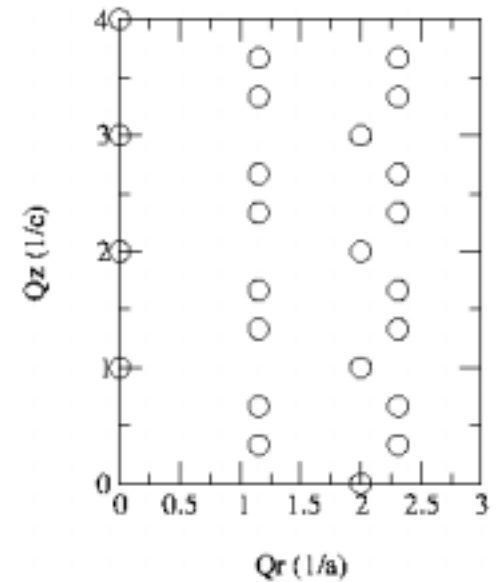
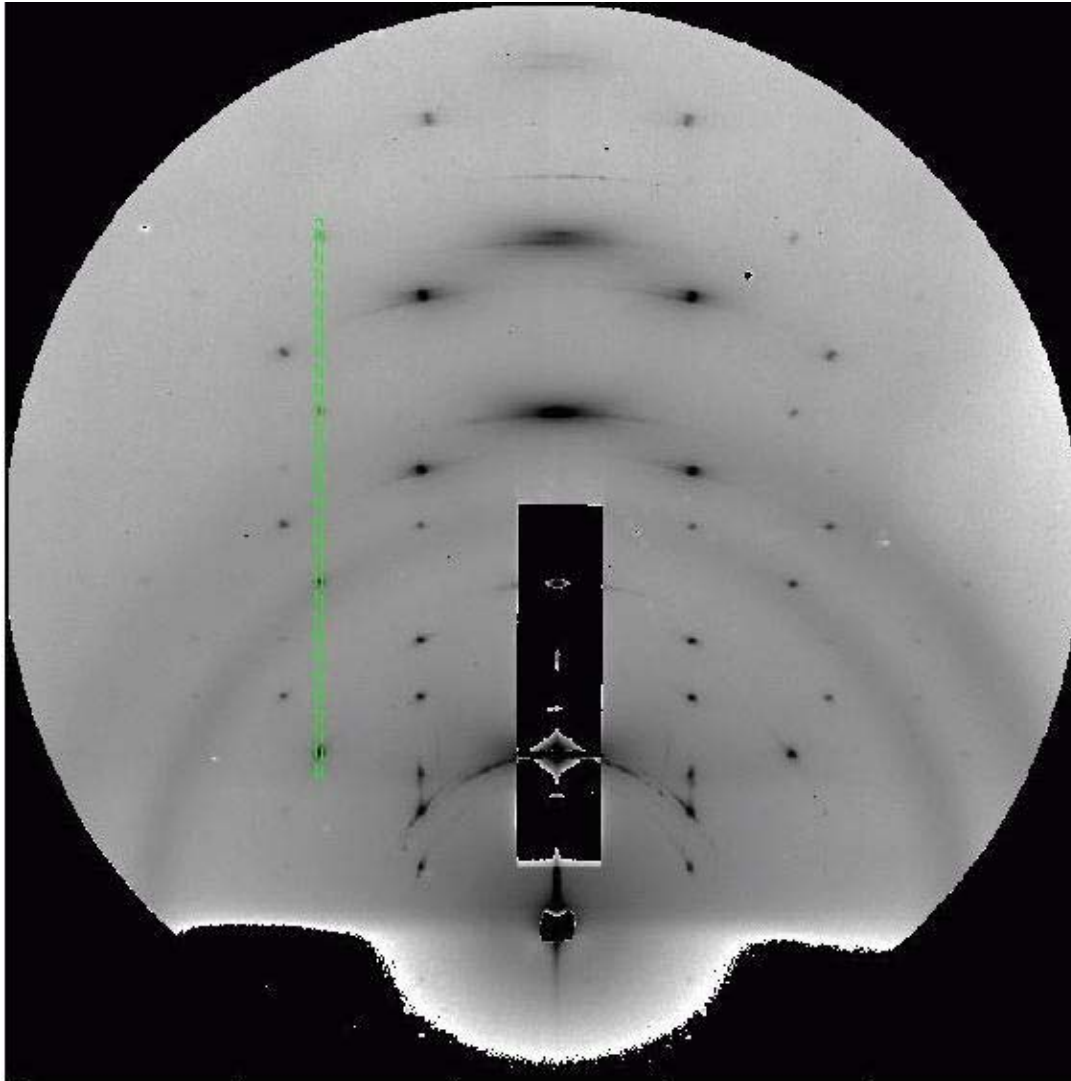
Yang et al., Biophys.
J. 77, 2648 (1999)

From uncorrelated to long-range correlated.



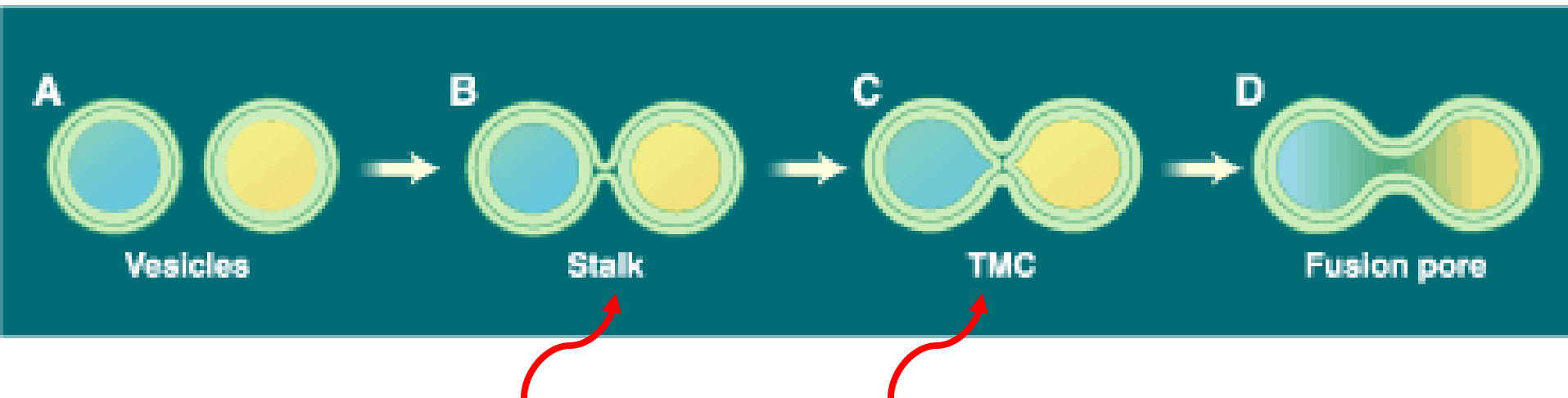
Rhombohedral lattice of pores

Yang et al., Biophys. J.
79, 2002 (2000)



Hexagonal ABC
stacking

(4) Membrane Fusion Problems

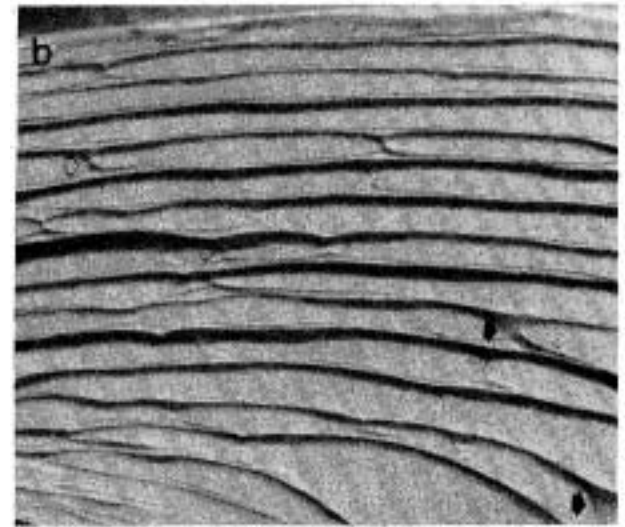
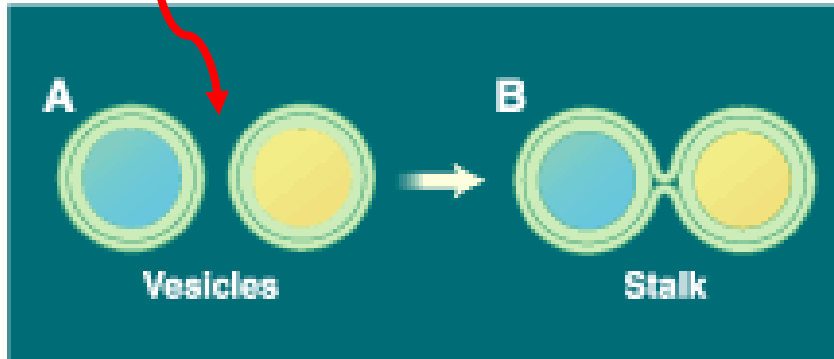


Are these the intermediate states? Do peptides affect these states?

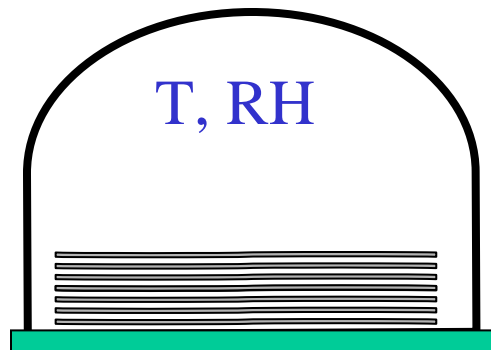
*This problem is related to gene delivery, drug delivery, and anti-viral drugs.

The first step of fusion

Removing the water molecules in between.



Hui et al., Science 212, 921 (1981)

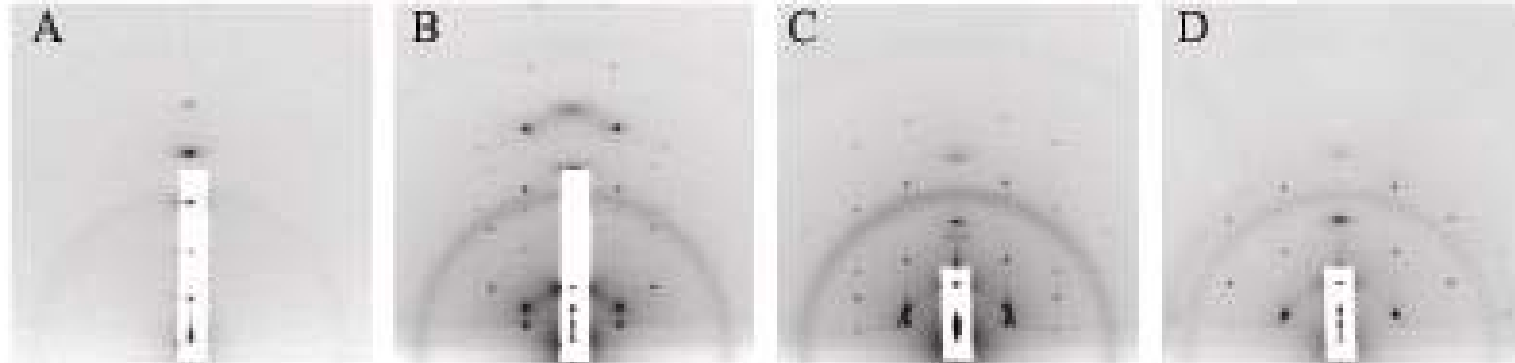


Reducing RH



DOPC/DOPE Mixtures

→ *RH*



Lamellar

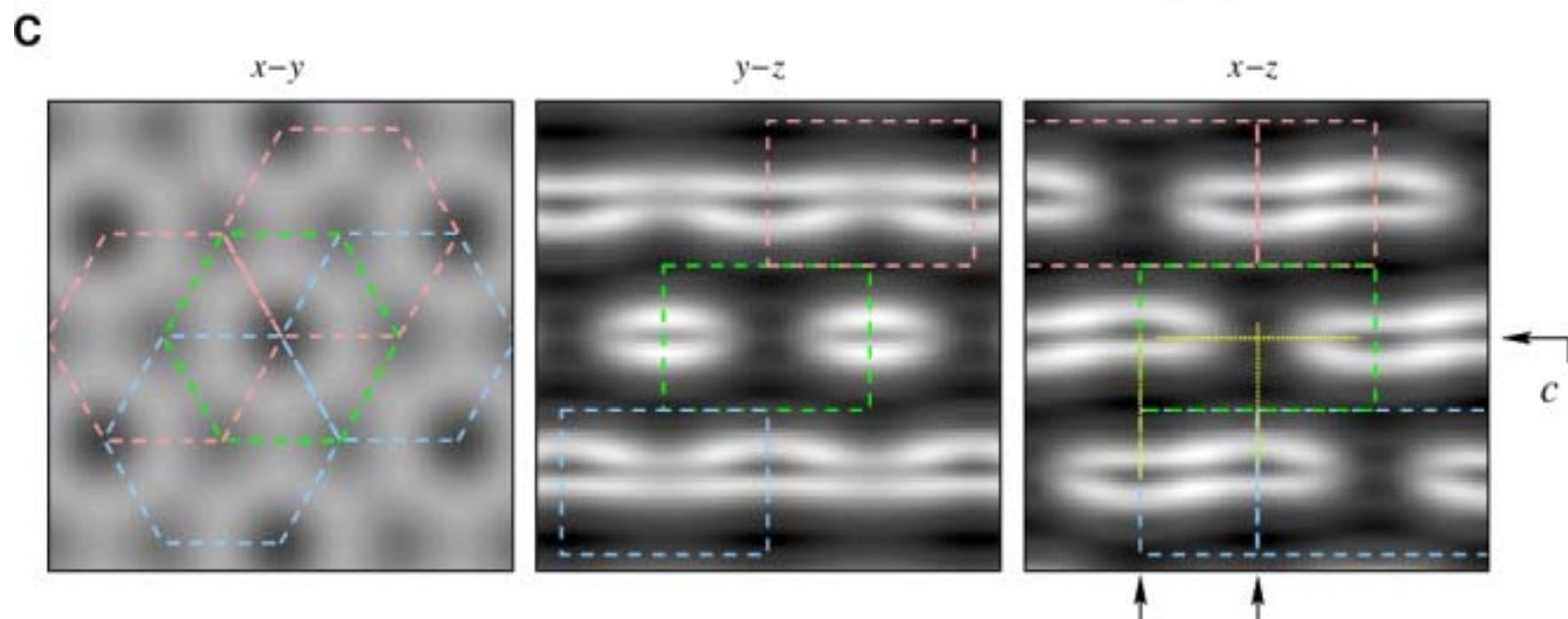
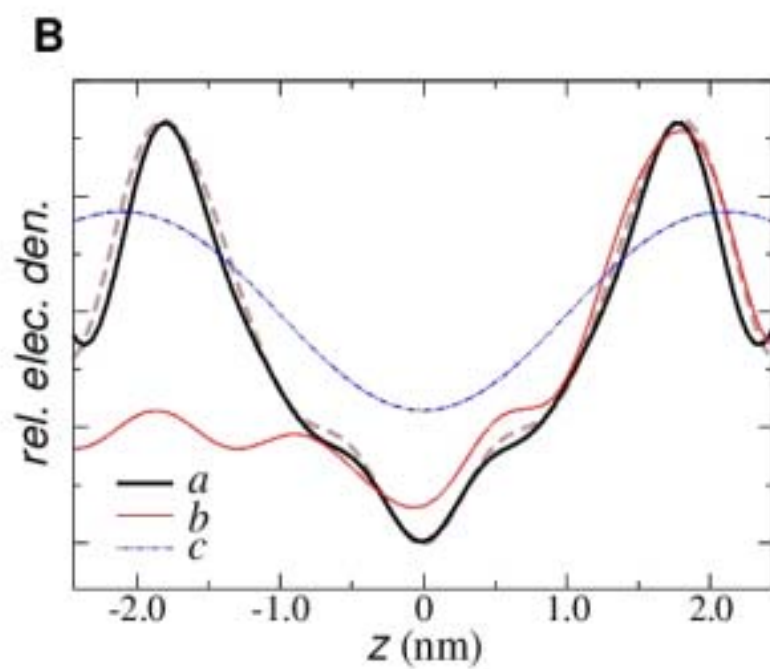
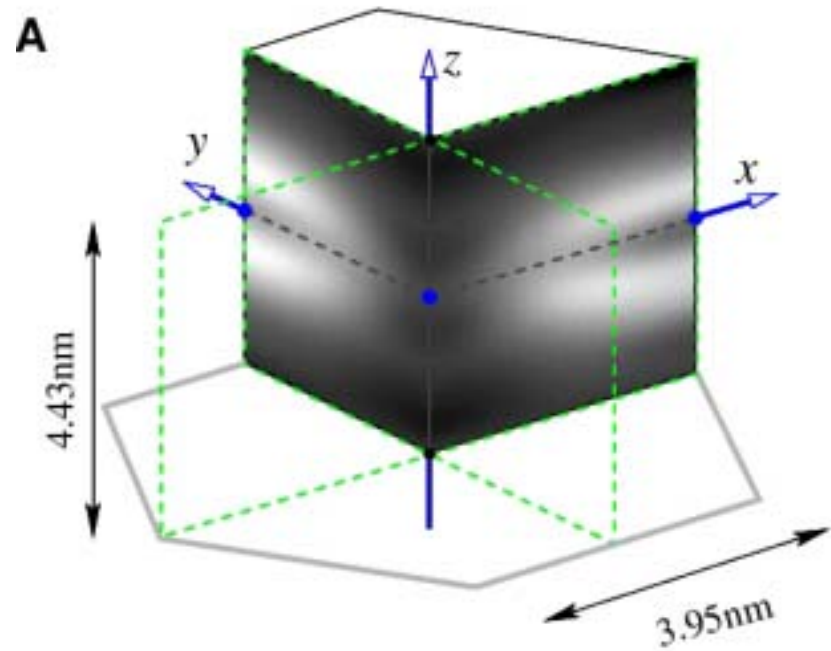
Rhombohedral

2D Monoclinic
(Distorted
Hexagonal)

Hexagonal

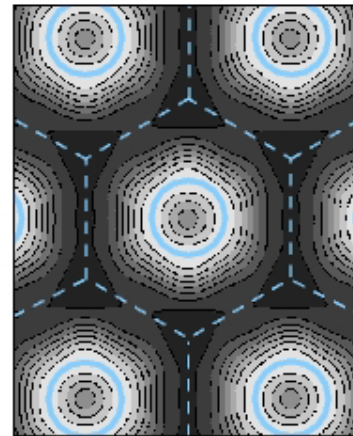
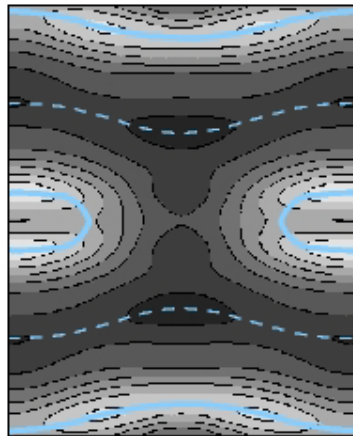
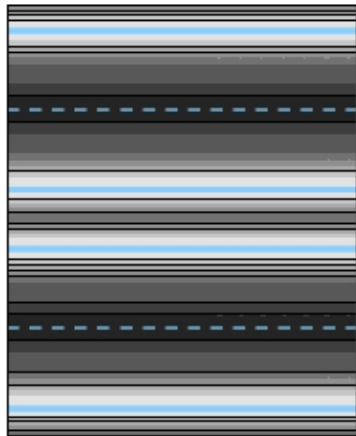
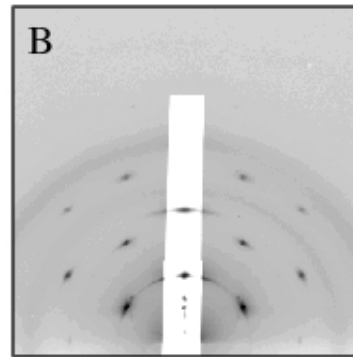
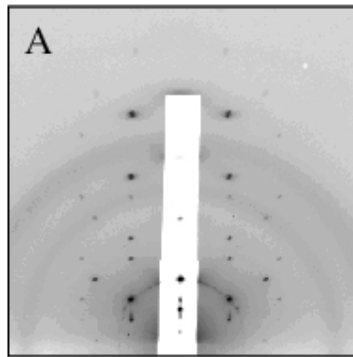
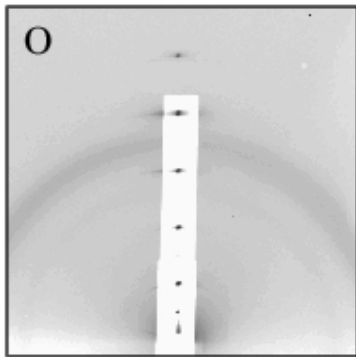
discovered in the last two years from
the substrate-supported samples.

Yang et al.,
Biochemistry 42,
6631 (2003)

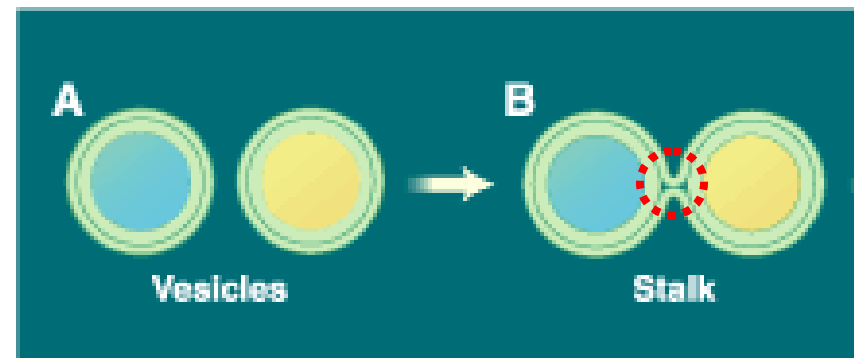
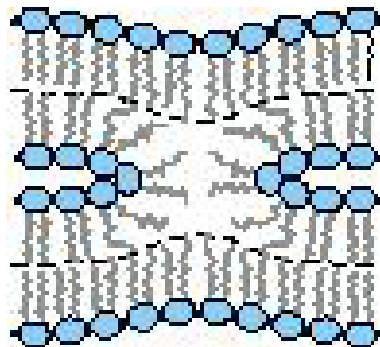


2D view

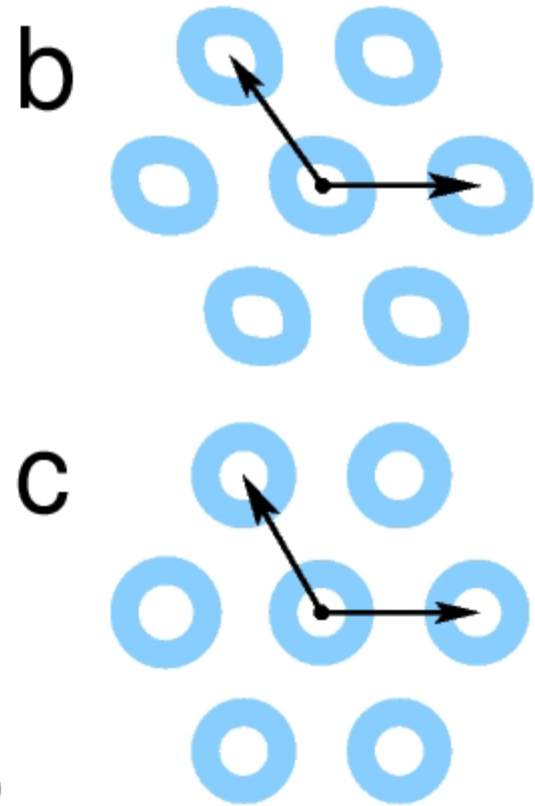
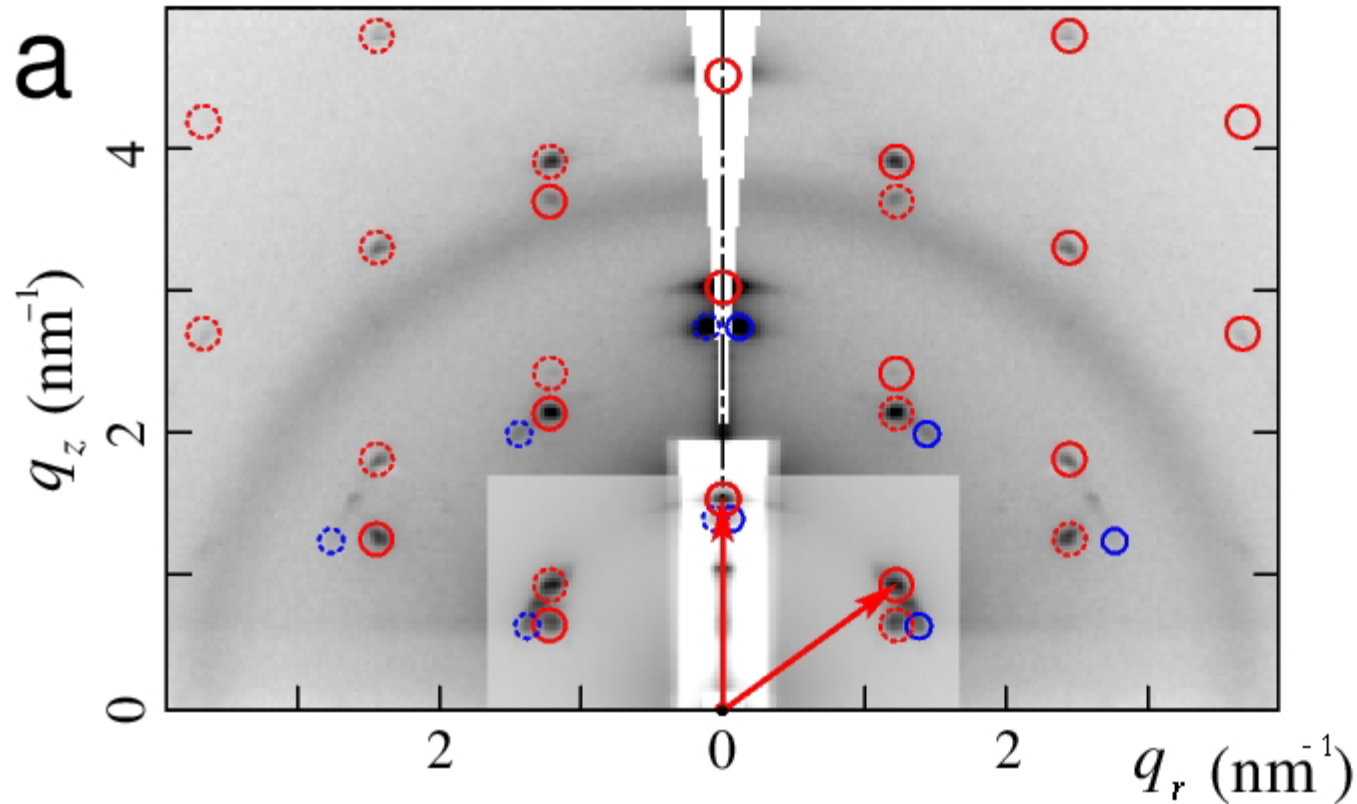
Yang and Huang,
Science 297, 1877
(2002)



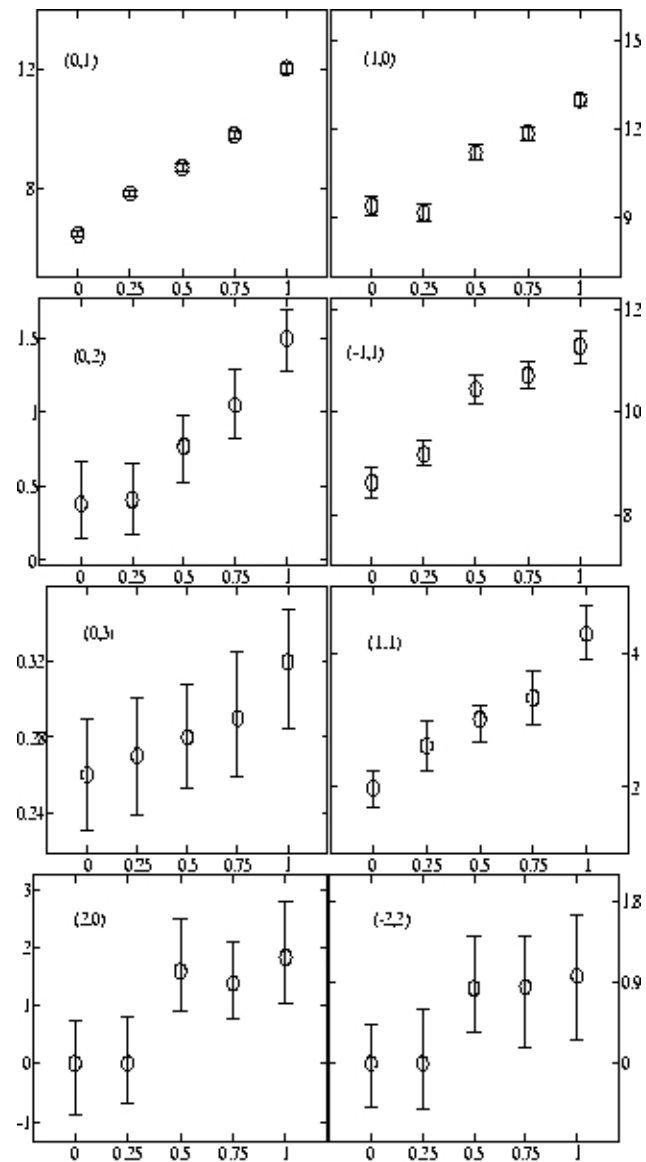
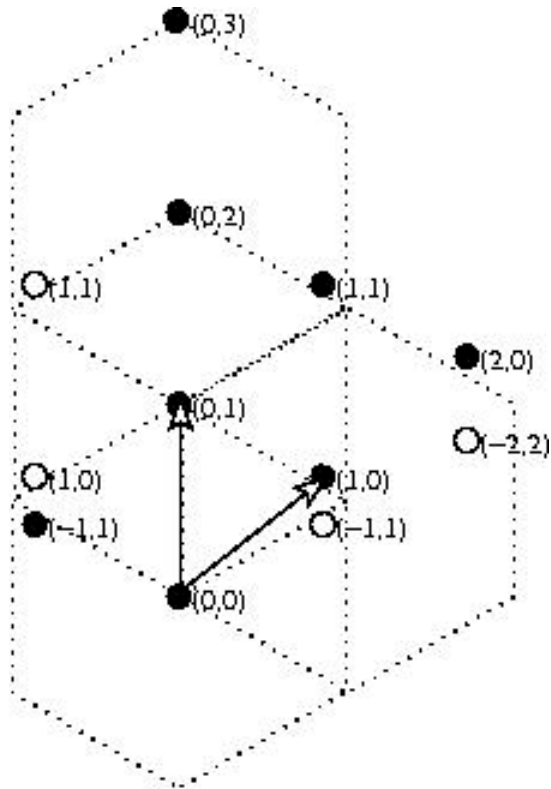
This experiment
validates the stalk
hypothesis for
membrane fusion.



Distorted Hexagonal Phase

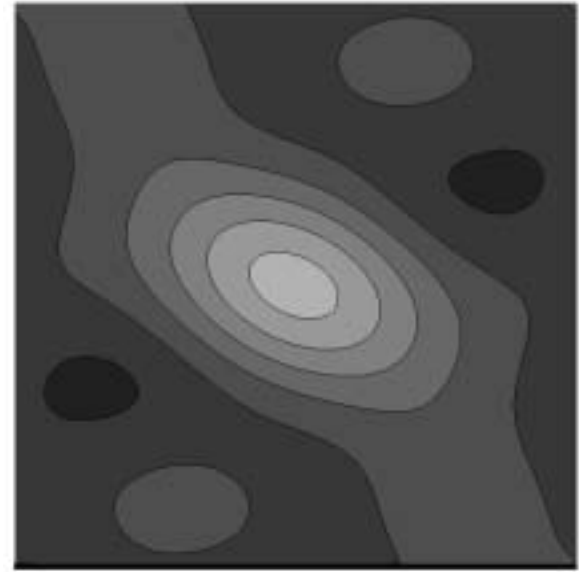
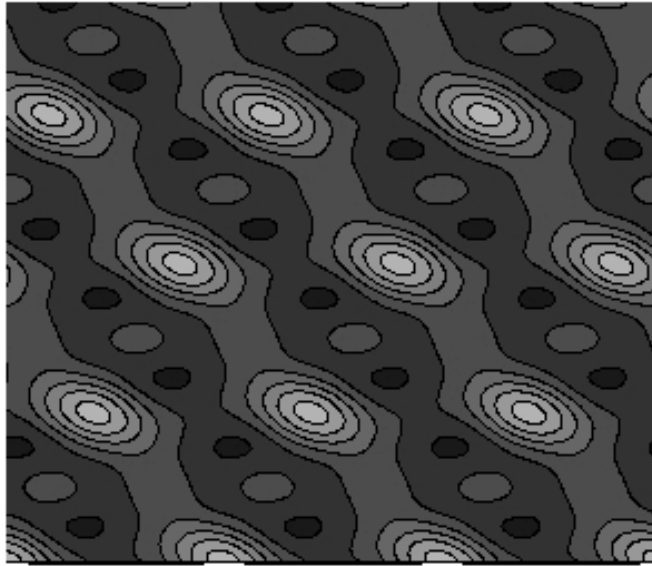


A_{DH} vs. x

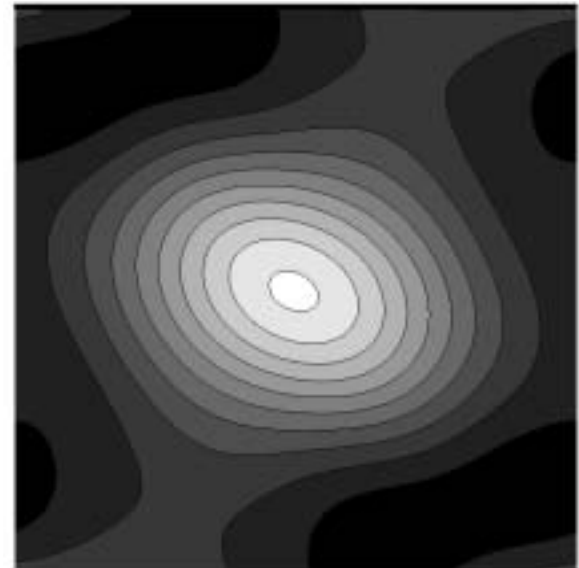
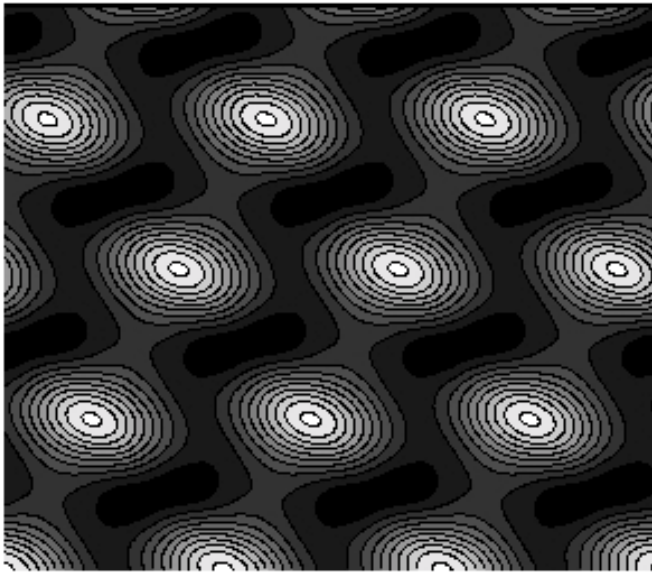


Water distribution vs. lipid distribution

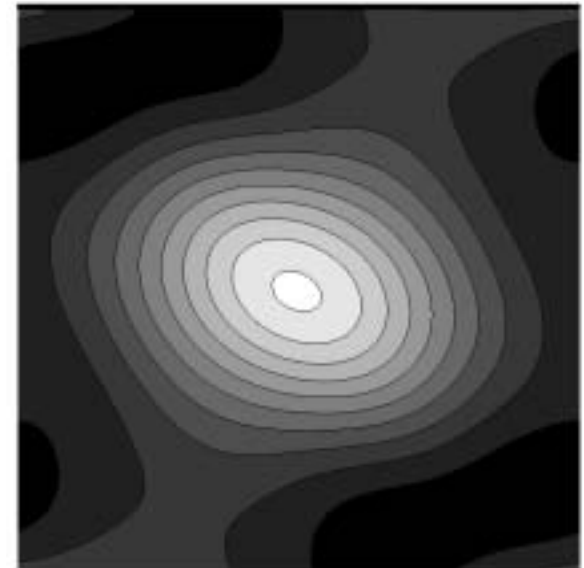
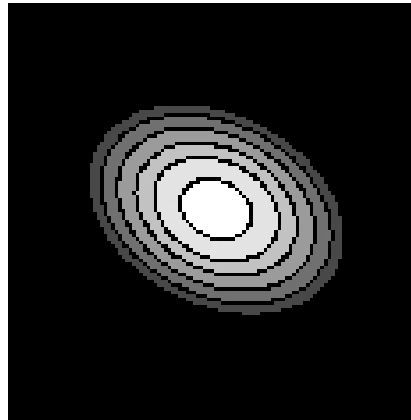
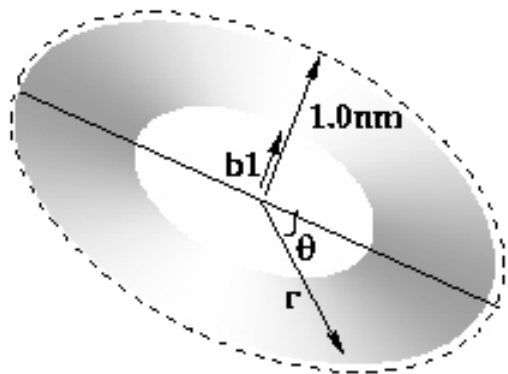
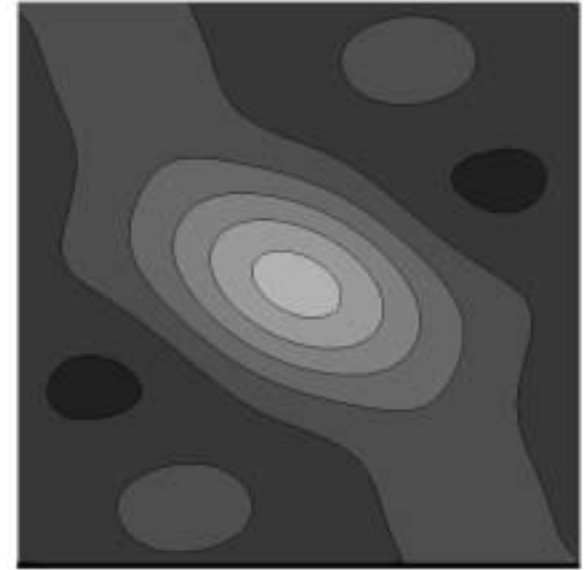
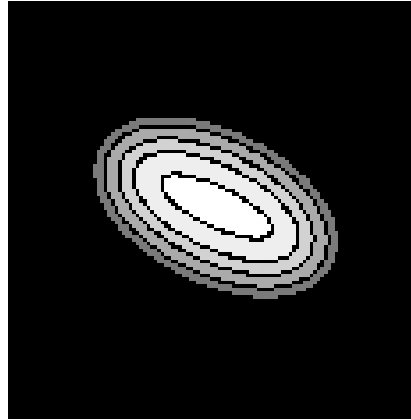
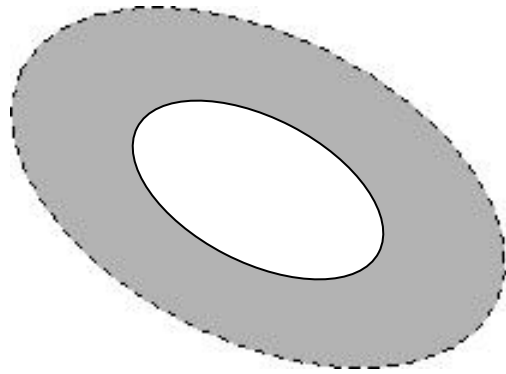
Water



Lipid

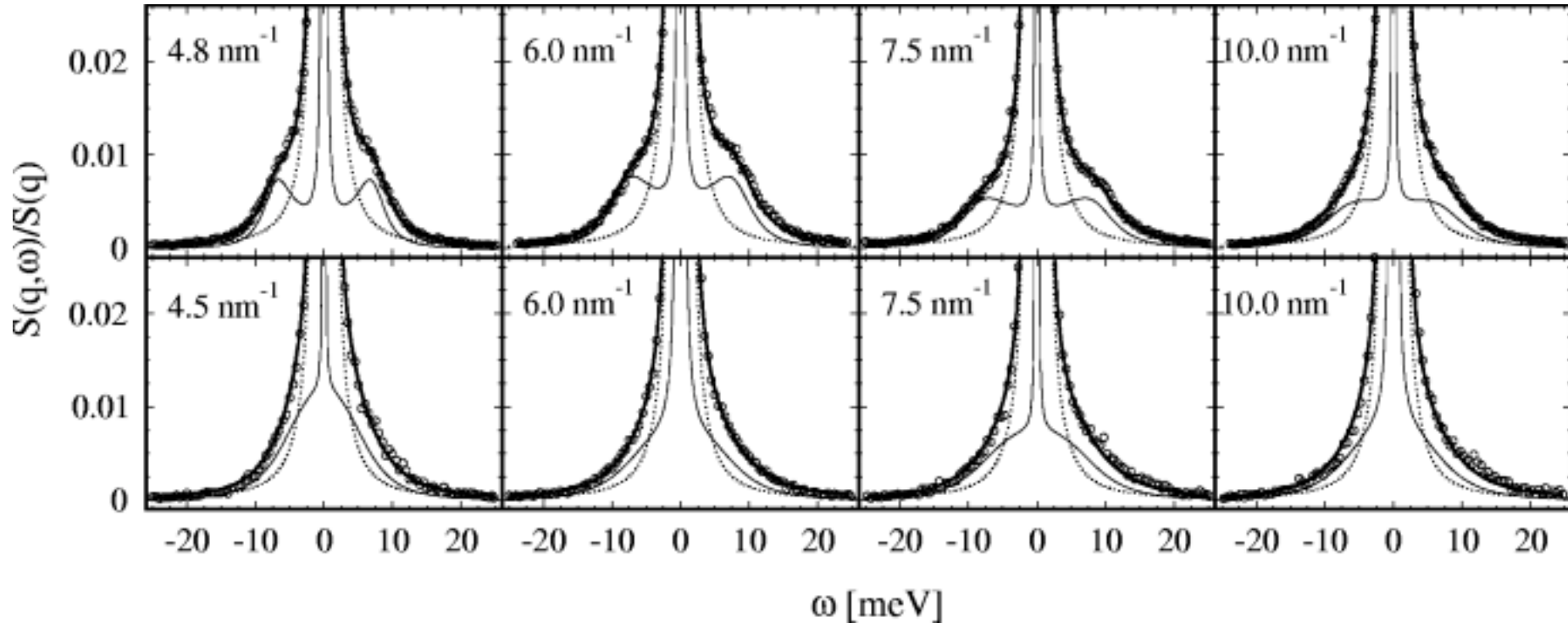


A bent monolayer of a lipid mixture demixes its components in reaching the minimum of free energy.

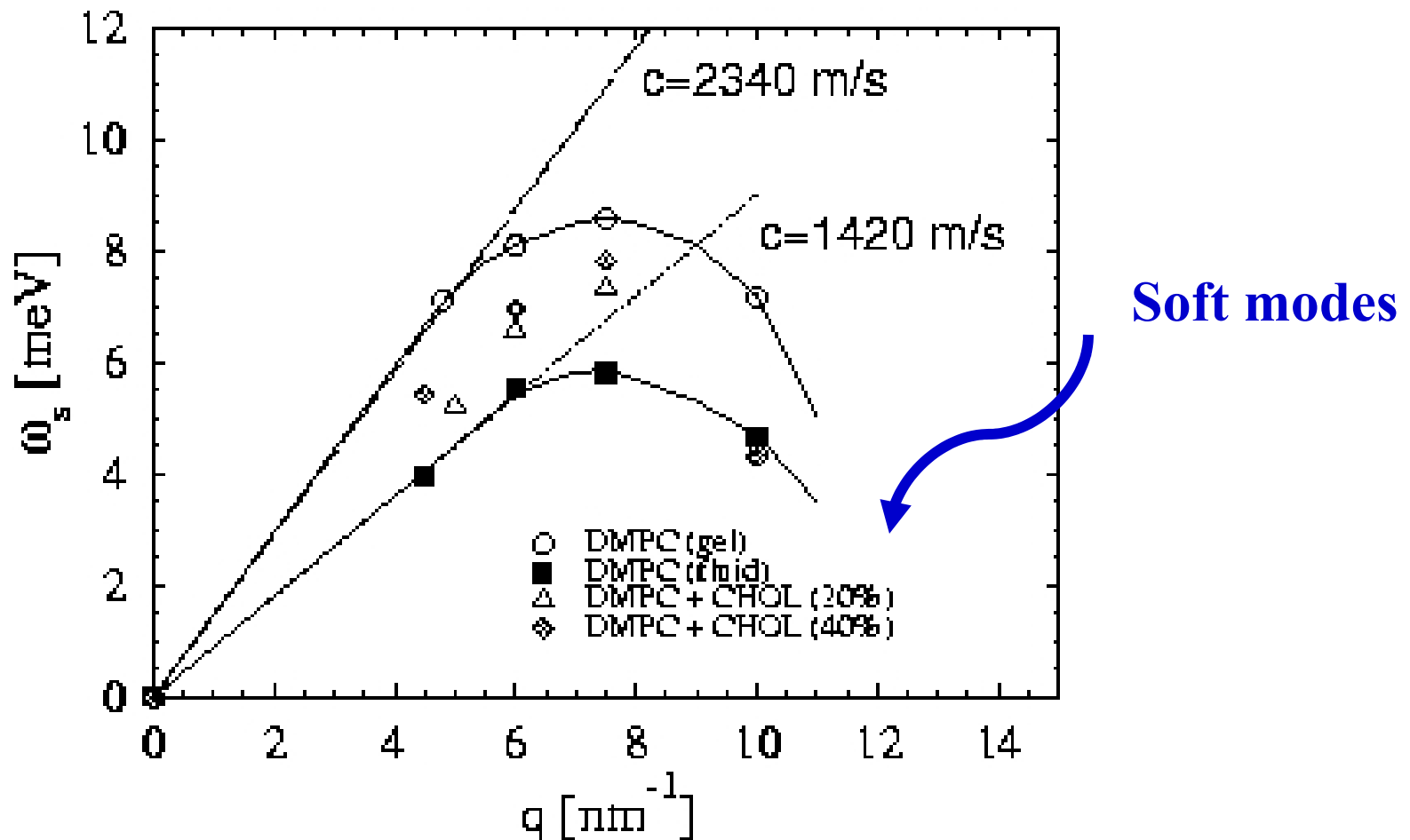


(5) Collective chain dynamics by in-plane inelastic X-ray scattering

Weiss et al., Biophys. J. 84, 3767 (2003)

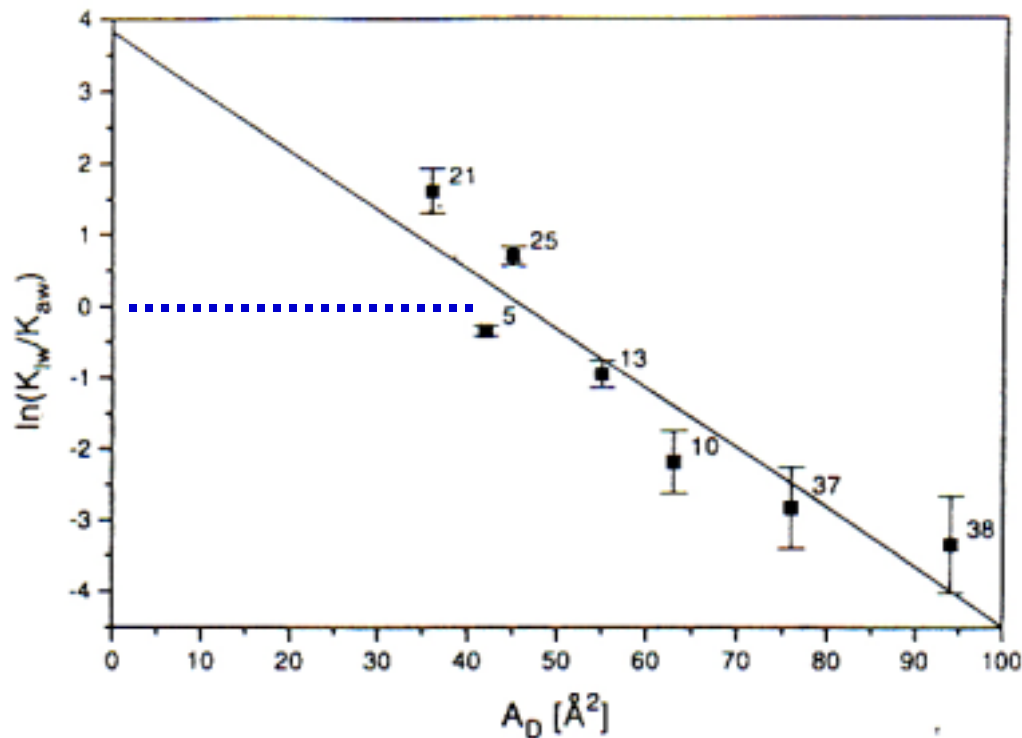


Dispersion curves of the sound mode propagating in-plane



The soft modes imply that It requires no work to insert a small peptide in the bilayer interface as shown by Seelig's experiment.

$$\ln(K_{lw}/K_{aw}) = -A_D\pi/k_B T$$



Fischer et al., J. Membr. Biol. 165, 201 (1998)

CONCLUSION

Synchrotron radiation is essential for membrane research. Two features are particularly desirable:

- 1) Energy tunable (5-15keV) GISAX beamline.
- 2) 0.5meV-resolution inelastic beamline.

Whodunit

Former and current Graduate Students:

Glenn Olah

Yili Wu

Ke He

Steve Ludtke

Thad Harroun

William Heller

Lin Yang

Thomas Weiss

Lai Ding

Wangchen Wang

Deng Pan

Yang Song

Collaborators:

Fang-Yu Chen, NCU

Ming-Tao Lee, NCU

Wei-Ching Hong, NCU

Bob Lehrer, UCLA

Alan Waring, UCLA

Dave Worcester, UM,
Columbia

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